METHODS AND COMPOSITIONS IN TREATING PAIN AND PAINFUL DISORDERS USING 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 OR 32620

Related Applications

[0001] The present application claims the benefit of U.S. Provisional Application Serial No. 60/444,781, filed on February 4, 2003, of U.S. Provisional Application Serial No. 60/452,291, filed on March 5, 2003, of U.S. Provisional Application Serial No. 60/454,540, filed on March 13, 2003, of U.S. Provisional Application Serial No. 60/478,805, filed on June 16, 2003 and of U.S. Provisional Application Serial No. 60/491,048, filed on July 30, 2003. The entire contents of these provisional patent applications are hereby incorporated in their entirety by this reference.

Background of the Invention

[0002] The sensation of pain can be categorized into two types, peripheral and central pain. Peripheral pain can be classified into three broad areas, nociceptive pain, inflammatory pain and neuropathic pain. Nociceptive pain is also referred to as physiological pain and serves as a defense mechanism throughout the animal kingdom. Inflammatory pain, arising from severe wounds and/or associated with inflammatory infiltrates, can be well controlled by non-steroidal anti-inflammatory drugs (NSAID)-like drugs, steroids and opiates. However, the etiology and management of neuropathic pain is not well understood. Neuropathic pain is thought to arise from inherent defects in sensory and as a consequence in sympathetic neurons and can be secondary to trauma.

[0003] Peripheral pain is mediated by two types of primary sensory neuron classes, the Ad- and C-fibers, whose cell bodies lie within the dorsal root ganglion. Although the mechanisms of generation of neuropathic pain are poorly understood it is clear that several factors influence the perception and transmission of the painful stimulus, namely, alterations in chemical environment, ectopic generation of sensory neuron firing and sympathetic discharge. Some of the most common syndromes associated with neuropathic pain arise from destruction of small sensory fibers (or possibly the alteration in ratios of small to large

fibers) as it is common in post-traumatic situations. Other etiologies of pain arise from small fiber damage due to diabetic neuropathy, drug induced damage (chemotherapy drugs), alcoholism, damage due to cancer, and a variety of hereditary small- and large-fiber neuropathies. We rationalize that targets derived from the peripheral nervous system may be of strategic benefit in that candidate compounds do not need to cross the blood-brain barrier, they can act on the initiation site of pain without inducing central side effects.

[0004] It has long been established that central mechanisms are involved in the perception and modulation of pain. Electrical stimulation of the periaqueductal gray (PAG) area produces analgesia without loss of other sensory modalities. Descending pain pathways emanating from PAG and the nucleus raphe magnus impinge on dorsal spinal cord regions where primary nociceptive afferents terminate. Also, stimulation of regions such as the paragigantocellularis nucleus in the medulla oblongata result in analgesia. Finally, opiate receptors, when stimulated by opioid alkaloids and opioid peptides, mediate analgesia and these sites are located in key "pain centers" within the brain including PAG, thalamic nuclei and cortical regions. Identification of genes in these CNS regions and the spinal thalamic tract from animal models of pain may elucidate important targets for pain modulation.

Detailed Description of the Invention

[0005] The present invention provides methods and compositions for the diagnosis and treatment of a subject experiencing pain or suffering from a painful disorders. Preferably, the subject is a human, e.g., a patient with pain or a pain-associated disorder disclosed herein. For example, the subject can be a patient with pain elicited from tissue injury, e.g., inflammation, infection, ischemia; pain associated with musculoskeletal disorders, e.g., joint pain; tooth pain; headaches, e.g., migrane; pain associated with surgery; pain related to inflammation, e.g., irritable bowel syndrome; or chest pain. The subject can be a patient with complex regional pain syndrome (CRPS), reflex sympathetic dystrophy (RSD), causalgia, neuralgia, central pain and dysesthesia syndrome, carotidynia, neurogenic pain, refractory cervicobrachial pain syndrome, myofascial pain syndrome, craniomandibular pain dysfunction syndrome, chronic idiopathic pain syndrome, Costen's pain-dysfunction, acute chest pain syndrome, gynecologic pain syndrome, patellofemoral pain syndrome, anterior knee pain syndrome, recurrent abdominal pain in children, colic, low back pain syndrome, neuropathic pain, phantom pain from amputation, phantom tooth pain, or pain asymbolia. The subject can be a cancer patient, e.g., a patient with brain

cancer, bone cancer, or prostate cancer. In other embodiments, the subject is a non-human animal, e.g., an experimental animal, e.g., an arthritic rat model of chronic pain, a chronic constriction injury (CCI) rat model of neuropathic pain, or a rat model of unilateral inflammatory pain by intraplantar injection of Freund's complete adjuvant (FCA).

[0006] "Treatment", as used herein, is defined as the application or administration of a therapeutic agent to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient, who has a disease or disorder, a symptom of disease or disorder or a predisposition toward a disease or disorder, with the purpose of curing, healing, alleviating, relieving, altering, remedying, ameliorating, improving or affecting the disease or disorder, the symptoms of disease or disorder or the predisposition toward a disease or disorder. A therapeutic agent includes, but is not limited to, the small molecules, peptides, antibodies, ribozymes and antisense oligonucleotides described herein.

[0007] The present invention is based, at least in part, on the discovery that nucleic acid and protein molecules, (described infra), are differentially expressed in animal models of pain and in peripheral and central nervous system tissues known to be associated with pain (e.g. dorsal root ganglion (DRG)). The modulators of the molecules of the present invention, identified according to the methods of the invention can be used to modulate (*e.g.,* inhibit, treat, or prevent) pain and painful conditions.

[0008] "Differential expression", as used herein, includes both quantitative as well as qualitative differences in the temporal and/or tissue expression pattern of a gene. Thus, a differentially expressed gene may have its expression activated or inactivated in normal versus painful disease conditions (for example, in an experimental pain model system such as in an animal model for pain). The degree to which expression differs in normal versus treated or control versus experimental states need only be large enough to be visualized via standard characterization techniques, *e.g.*, quantitative PCR, Northern analysis, subtractive hybridization. The expression pattern of a differentially expressed gene may be used as part of a prognostic or diagnostic, evaluation, or may be used in methods for identifying compounds useful for the treatment of pain and painful disorders. In addition, a differentially expressed gene involved in pain or painful disorders may represent a target gene such that modulation of the level of target gene expression or of target gene product activity may act to ameliorate a painful disease condition. Compounds that modulate target gene expression or activity of the target gene product can be used in the treatment of pain or painful conditions. Although the genes described herein may be differentially expressed

with respect to pain, and/or their products may interact with gene products important to pain, the genes may also be involved in mechanisms important to additional cell processes.

Molecules of the Present Invention

[0009] Molecules of the present invention include, but are not limited to ion channels (*e.g.* Potassium channels), transporters (e.g. amino acid transporters), receptors (e.g. G protein coupled receptors) and enzymes (e.g. kinases)

[0010] Transmembrane ion channel proteins that selectively mediate the conductance of sodium, potassium, calcium and chloride ions directly modulate the electrical activity of sensory neurons and are, thus, important in nociception. In particular, potassium channels are main players in regulating the frequency and pattern of neuronal firing. The expression and peak currents of potassium channels has been shown to be regulated after different models of inflammatory and chronic pain. Additionally, calcium ions serve important intracellular signaling roles including modulation of other ion channels and regulation of protein kinases and other enzymatic activity. As cell surface proteins with established three-dimensional structures and modes of action, the pore-forming alpha subunits of ion channels make ideal drug targets. In addition to alpha subunits, these channels may consist of beta subunits and other interacting proteins which modulate channel activity and are good targets for pharmacological manipulation of the channels. Therefore, ion channels are useful in treating pain and painful conditions.

[0011] Endogenous soluble factors mediate pain sensation by binding to specific transmembrane receptors either on the peripheral terminals of nociceptive neurons or on central neurons receiving input from these nociceptors. These soluble factors include, but are not limited to serotonine, histamine, bradykinin, tachykinins (substance P and neurokinin A), opioids, eicosanoids (leukotrienes, prostaglandins, thromboxanes), purines, excitatory amino acids and different proteins. In addition a growing body of evidence, including clinical trials in man, indicates that IL-1, TNFa, and members of the neurotrophin family are involved at several stages in the transmission of painful stimuli. Hydrogen ions (protons) may mediate pain associated with inflammation (and also acid taste) by activating vanilloid receptor calcium channels or amiloride-sensitive sodium channels. Additionally, numerous exogenous agents modulate pain by mimicking endogenous soluble factors. For instance the opiate drugs of abuse exert analgesic effects by binding to receptors for the endogenous opioids and capsaicin stimulates pain sensation by binding to vanilloid receptors. The receptors for these soluble factors are linked to several signal transduction mechanisms

including tyrosine kinase activity (e.g. neurotrophin receptors), recruitment of cytoplasmic tyrosine kinases (e.g. cytokine receptors for TNFa and IL-1), ion channel opening, and G-protein coupled receptors. These cell surface receptors are ideal drug targets due to their transmembrane location, and the goal is to discover G-protein coupling receptors with known ligands or with surrogate ligands that may be important players in regulating pain mechanisms.

[0012] Intracellular kinases such as protein kinase A and protein kinase C are involved in the response to pain in sensory neurons. Similarly, enzymes such as cyclooxygenase(s) and thromboxane synthetase are know to be critical in the production of prostaglandins, leukotrienes and thromboxanes. Although these particular targets may be more important in inflammatory pain, the role of this gene family in long term or neuropathic pain is of importance.

Gene ID 16386

[0013] The human 16386 sequence (SEQ ID NO:1), known also as Brain sulfotransferase-like protein (hBR-STL), is approximately 2419 nucleotides long including untranslated regions. The coding sequence, located at about nucleic acid 21 to 875 of (SEQ ID NO:1), encodes a 284 amino acid protein (SEQ ID NO:2).

[0014] As assessed by TaqMan analysis, 16386 mRNA was primarily expressed in tissues of the central nervous system (CNS) in rat and human tissue samples. Additionally, 16386 mRNA was shown to be up-regulated in dorsal root ganglion (DRG) samples after chronic constriction injury (CCI). 16386 mRNA was also shown to be down-regulated in spinal cord samples after Rhizotomy. 16386 mRNA showed a 2 fold up-regulation in the DRG after Axotomy, Complete Freund's Adjuvant (CFA), Capsaicin, Spared Nerve Injury (SNI), or Tibial Nerve Injury (TNI). In addition, 16386 mRNA also showed a 2 fold up-regulation in spinal cord after Axotomy, CFA, capsaicin, SNI, TNI or CCI.

[0015] 16386 is a sulfotransferase which acts on tyrosine derivative compounds. 16386 is potentially responsible for the inhibition and removal of dopamine by sulfation. Dopamine has analgesic effects in the spinal cord and central nervous system. Thus, inhibition of 16386 potentially has analgesic effects and thus is a novel inhibitor of pain. Due to 16386 expression in the dorsal root ganglion and spinal cord, along with its functional role, modulators of 16386 activity would be useful in treating disorders associated

with the treatment of pain and painful disorders. 16386 polypeptides of the present invention are useful in screening for modulators of 16386 activity.

Gene ID 15402

[0016] The human 15402 sequence (SEQ ID NO:3), known also as HNK1 Sulfotransferase, is approximately 2877 nucleotides long including untranslated regions. The coding sequence, located at about nucleic acid 387 to 1457 of (SEQ ID NO:3), encodes a 356 amino acid protein (SEQ ID NO:4).

[0017] As assessed by TaqMan analysis, 15402 mRNA was primarily expressed in tissues of the central nervous system in both human and rat panels. 15402 mRNA was strongly up-regulated in the dorsal root ganglion (DRG) after Chronic Constriction Injury (CCI) and Axotomy. 15402 mRNA was down-regulated in the DRG after Rhizotomy and in the spinal cord after Tibial Nerve Injury (TNI).

[0018] 15402 transfers a sulfate to the HNK-1 carbohydrate recognition site present in myelin-associated glycoprotein (MAG). Sulfated MAG is a major target for monoclonal IgM in patients with demyelinating neuropathy. Thus, inhibition of 15402 potentially blocks antibody interaction with MAG and thus could inhibit pain caused by demyelinating neuropathy. Due to the expression of 15402 in the spinal cord and dorsal root ganglion, along with its functional role, modulators of 15402 activity would be useful in treating disorders associated with the treatment of pain and painful disorders. 15402 polypeptides of the present invention are useful in screening for modulators of 15402 activity.

Gene ID 21165

[0019] The human 21165 sequence (SEQ ID NO:5), known also as lactosylceramide (LacCer) synthase, is approximately 3931 nucleotides long including untranslated regions. The coding sequence, located at about nucleic acid 298 to 1446 of (SEQ ID NO:5), encodes a 382 amino acid protein (SEQ ID NO:6).

[0020] As assessed by TaqMan analysis, 21165 mRNA was primarily expressed in tissues of the central nervous system in both human and rat panels. 21165 mRNA was strongly up-regulated in dorsal root ganglion (DRG) after Chronic Constriction Injury (CCI). 21165 mRNA was down-regulated in the DRG and spinal cord after Tibial Nerve Injury (TNI) and long-term Capsaicin treatment. 21165 mRNA was also down-regulated in DRG after Spared Nerve Injury (SNI), Complete Freund's Adjuvant (CFA) and Rhizotomy.

[0021] Inhibition of 21165 or lactosylceramide (LacCer) synthase, inhibits NGF pathways. In addition, inhibition of 21165 inhibits neuronal function. 21165 levels are markedly increased in a variety of peripheral neuropathies, and exogenous 21165 activates NF-Kappa-B pathways known to be involved in pain mediation. Exogenous 21165 also induces superoxide production and ERK-1, both of which have been demonstrated to be up-regulated in a variety of pain models. Finally, 21165 synthesis is up-regulated by TNF-alpha, a well established mediator of neuropathic pain, and this up-regulation results in an increase of ICAM-1, which has been shown to be up-regulated in a variety of painful conditions in both humans and animals. Thus, in a variety of manners, inhibitors of 21165 will likely be a novel mechanism for pain inhibition. Due to the expression of 21165 in the spinal cord and dorsal root ganglion, along with its functional role, modulators of 21165 activity would be useful in treating disorders associated with the treatment of pain and painful disorders. 21165 polypeptides of the present invention are useful in screening for modulators of 21165 activity.

Gene ID 1423

[0022] The human 1423 sequence (SEQ ID NO:7), known also as a N-Methyl-D-Aspartic Acid (NMDA) receptor, is approximately 3924 nucleotides long including untranslated regions. The coding sequence, located at about nucleic acid 1 to 3168 of (SEQ ID NO:7), encodes a 1055 amino acid protein (SEQ ID NO:8).

[0023] As assessed by TaqMan analysis, 1423 mRNA was primarily expressed in tissues of the central nervous system in both human and rat panels. 1423 mRNA was up-regulated in dorsal root ganglion (DRG) after chronic constriction injury (CCI) and in spinal cord after axotomy.

[0024] NMDA receptor activation is one of the basic mechanisms responsible for pain responses. Modulating the activity of NMDA receptors by antagonizing the activation of ephrin B2 (ephB2) may, not only block pain responses, but also block the synaptic plasticity involved in the maintenance of pain behaviors. Due to the expression of 1423 mRNA in the spinal cord and dorsal root ganglion, along with its functional role, modulators of 1423 activity would be useful in treating disorders associated with the treatment of pain and painful disorders. 1423 polypeptides of the present invention are useful in screening for modulators of 1423 activity.

Gene ID 636

[0025] The human 636 sequence (SEQ ID NO:9), known also as Potassium voltage-gated channel subfamily A member 6 (KCNA6); Potassium channel Kv1.6; or HBK2 potassium channel, is approximately 4234 nucleotides long including untranslated regions. The coding sequence, located at about nucleic acid 863 to 2452 of SEQ ID NO:9, encodes a 529 amino acid protein (SEQ ID NO:10).

[0026] As assessed by TaqMan analysis, 636 mRNA was primarily expressed in tissues of the central nervous system in rat and human panels. 636 mRNA was up-regulated in the dorsal root ganglion (DRG) and spinal cord (SC) after Tibial Nerve Injury (TNI). 636 mRNA was down-regulated in spinal cord (SC) after rhizotomy and in the dorsal root ganglion (DRG) after Capsaicin treatment. *In situ* hybridization experiments indicated that 636 mRNA was expressed in the spinal cord, brain, and a subpopulation of DRG neurons in both monkey and rat tissues.

[0027] Activation of potassium channels affects the frequency and the pattern of neuronal firing. Several voltage-gated potassium channels are expressed in sub-population of sensory neurons including those involved in nociception. In general, it has been shown that the expression of some voltage-gated potassium channels decreases in DRG neurons after axotomy and that the peak of potassium currents is reduced in sensory neurons during chronic inflammation. Furthermore, administration of potassium channel openers potentiated the antinociception produced by agonists of alpha-2-adrenoreceptors or by morphine. Due to 636 mRNA expression in the dorsal root ganglion and spinal cord, along with its functional role, modulators of 636 activity would be useful in treating disorders associated with the treatment of pain and painful disorders. 636 polypeptides of the present invention are useful in screening for modulators of 636 activity.

Gene ID 12303

[0028] The human 12303 sequence (SEQ ID NO:11), known also as Potassium channel KCNK4; TRAAK; or Two pore K+ channel KT4.1, is approximately 2747 nucleotides long including untranslated regions. The coding sequence, located at about nucleic acid 51 to 1310 of SEQ ID NO:11, encodes a 419 amino acid protein (SEQ ID NO:12).

[0029] As assessed by TaqMan analysis, 12303 mRNA was primarily expressed in tissues of the central nervous system (CNS) in both monkey and rat panels. 12303 mRNA

was up-regulated in dorsal root ganglion (DRG) after Chronic Constriction Injury (CCI) and in the spinal cord after Capsaicin treatment. 12303 mRNA was down-regulated in spinal cord after Rhizotomy. *In situ* hybridization experiments indicated that 12303 mRNA was present in most neurons of monkey and rat DRG's and spinal cord tissues.

[0030] Activation of potassium channels affects the frequency and the pattern of neuronal firing. Several voltage-gated potassium channels are expressed in sub-population of sensory neurons including those involved in nociception. In general, it has been shown that the expression of some voltage-gated potassium channels decreases in DRG neurons after axotomy and that the peak of potassium currents is reduced in sensory neurons during chronic inflammation. Furthermore, administration of potassium channel openers potentiated the antinociception produced by agonists of alpha-2-adrenoreceptors or by morphine. The antinociception induced by intrathecal injection of morphine.injection is blocked in a dose-dependent manner by glibenclamide (a blocker of ATP-sensitive K+ channels) and potentiated by nicorandil (a opener of ATP-sensitive K+ channels). 12303 (the potassium channel TRAAK) is important for the modulation of the firing pattern of nociceptive neurons. Drugs which open 12303 are potentially a novel mechanism for the inhibition of pain. Due to the expression of 12303 in the spinal cord and dorsal root ganglion, along with its functional role, modulators of 12303 activity would be useful in treating disorders associated with the treatment of pain and painful disorders. 12303 polypeptides of the present invention are useful in screening for modulators of 12303 activity.

Gene ID 21425

[0031] The human 21425 sequence (SEQ ID NO:13), known also as ELK2 potassium channel, is approximately 3985 nucleotides long including untranslated regions. The coding sequence, located at about nucleic acid 359 to 3610 of SEQ ID NO:13, encodes a 1083 amino acid protein (SEQ ID NO:14).

[0032] As assessed by TaqMan analysis, 21425 mRNA was expressed exclusively in tissues of the central nervous sytem (CNS) in both human and rat panels. 21425 mRNA was up-regulated in the dorsal root ganglion (DRG) after Chronic Constriction Injury (CCI), Tibial Nerve Injury (TNI) and Axotomy. 21425 mRNA was also up-regulated in spinal cord after Capsaicin, Spared Nerve Injury (SNI) and TNI. *In situ* hybridization experiments indicated that 21425 mRNA was highly expressed in monkey brain in a majority of neurons.

[0033] The human ELK2 K channel or 21425 is critical for hypersensitivity in different pain states and may therefore represent a unique target for pain. Due to the expression of 21425 in the spinal cord and dorsal root ganglion, along with its functional role, modulators of 21425 activity would be useful in treating disorders associated with the treatment of pain and painful disorders. 21425 polypeptides of the present invention are useful in screening for modulators of 21425 activity.

Gene ID 27410

[0034] The human 27410 sequence (SEQ ID NO:15), known also as Potassium channel subfamily K member 17 (KCNK17); TWIK-related alkaline pH activated K+ channel 2 (TALK-2); or TWIK-related acid-sensitive K+ channel 4 (TASK-4), is approximately 1764 nucleotides long including untranslated regions. The coding sequence, located at about nucleic acid 268 to 1266 of SEQ ID NO:15, encodes a 332 amino acid protein (SEQ ID NO:16).

[0035] As assessed by TaqMan analysis, 27410 mRNA was expressed at low levels in the lung, spinal cord, dorsal root ganglion (DRG) and liver. *In situ* hybridization experiments indicated that 27410 mRNA showed diffuse expression in human and rat brain, spinal cord and DRG tissues.

[0036] Activation of potassium channels affects the frequency and the pattern of neuronal firing. Several voltage-gated potassium channels are expressed in sub-population of sensory neurons including those involved in nociception. In general, it has been shown that the expression of some voltage-gated potassium channels decreases in DRG neurons after axotomy and that the peak of potassium currents is reduced in sensory neurons during chronic inflammation. Furthermore, administration of potassium channel openers potentiated the antinociception produced by agonists of alpha-2-adrenoreceptors or by morphine. The antinociception induced by intrathecal injection of morphine.injection is blocked in a dose-dependent manner by glibenclamide (a blocker of ATP-sensitive K+ channels) and potentiated by nicorandil (a opener of ATP-sensitive K+ channels). 27410 is important for the modulation of the firing pattern of nociceptive neurons. Drugs which open 27410 are novel mechanisms for the inhibition of pain. Due to the expression of 27410 in the spinal cord and dorsal root ganglion, along with its functional role, modulators of 27410 activity would be useful in treating disorders associated with the treatment of pain and

painful disorders. 27410 polypeptides of the present invention are useful in screening for modulators of 27410 activity.

Gene ID 38554

[0037] The human 38554 sequence (SEQ ID NO:17), known also as Solute carrier family 21 member 14 (SLC21A14); Organic anion transporter F (OATP-F); Organic anion transporting polypeptide 14 (OATP14); or Organic anion transporter polypeptide related protein 5 (OATPRP5), is approximately 3227 nucleotides long including untranslated regions. The coding sequence, located at about nucleic acid 345 to 2483 of SEQ ID NO:17, encodes a 712 amino acid protein (SEQ ID NO:18).

[0038] As assessed by TaqMan analysis, 38554 mRNA was expressed predominantly in tissues of the central nervous system (CNS) in both rat and human panels. 38554 mRNA was up-regulated in dorsal root ganglion (DRG) after Axotomy and down-regulated in DRG after Spared Nerve Injury (SNI), Tibial Nerve Injury (TNI) and long term capsaicin treatment. 38554 mRNA was also down-regulated in spinal cord after SNI (8 weeks). *In situ* hybridization experiments indicated that 38554 mRNA was expressed throughout the brain, spinal cord, choroid plexus and DRG tissues, particularly in glial cells.

[0039] 38554 is OATP-F, a member of the Organic Anion Transporter Polypeptide (OATP) family. OATP-F has been shown to transport estrone-3-sulfate and dihydroepiandrosterone sulfate. Other members of the OATP family, such as human OATP8, OATP-A and OATP-C, transport the opioid receptor agonists [D-penicillamine(2,5)]-enkephalin. OATP8 and OATP-A also transport the opioid receptor agonist deltorphin (*Gastroenterology 120:525; J Pharmacol Exp Ther 294(1):73*).

[0040] 38554 is a critical gene in pain because it is a member of a family of transporters which transports opioid receptor agonists that are known to be involved in pain transmission. Blockers of 38554 could increase endogenous opioid receptor compounds and therefore be novel mechanisms for pain inhibition. Due to the expression of 38554 in the spinal cord and dorsal root ganglion, along with its functional role, modulators of 38554 activity would be useful in treating disorders associated with the treatment of pain and painful disorders. 38554 polypeptides of the present invention are useful in screening for modulators of 38554 activity.

Gene ID 38555

[0041] The human 38555 sequence (SEQ ID NO:19), known also as Solute carrier family 21 member 11 (SLC21A11); Organic anion transporting polypeptide D (OATP-D); Organic anion transporter polypeptide related protein 3 (OATPRP3); or PGE1 transporterOAPT-D, is approximately 2133 nucleotides long. The coding sequence, located at about nucleic acid 1 to 2133 of SEQ ID NO:19, encodes a 710 amino acid protein (SEQ ID NO:20).

[0042] As assessed by TaqMan analysis, 38555 mRNA was expressed at high levels in tissues of the central nervous sysytem (CNS) in both rat and human panels. 38555 mRNA was up-regulated in the dorsal root ganglion (DRG) after Axotomy and in the spinal cord after Chronic Constriction Injury (CCI) and Tibial Nerve Injury (TNI). 38555 mRNA was down-regulated in DRG after Spared Nerve Injury (SNI) or TNI. *In situ* hybridization experiments indicated that 38555 mRNA was expressed in brain, spinal cord, heart, small and medium sized neurons in DRG and sympathetic neurons.

[0043] 38555 is OAPT-D, a member of the Organic Anion Transporter Polypeptide (OATP) family. OATP-D has been shown to transport estrone-3-sulfate, benzylpenicillin, and Prostaglandin E2 (*Biochem Biophys Res Commun 273:251*). Prostaglandin E2 has been shown to mediate bradykinin-induced hyperalgesia caused by intradermal injection of adenosine in the hindpaw of the rat (*Neuroscience 1990;38(3):757-62*). Other members of the OATP family (human OATP8, OATP-A and OATP-C) transport the opioid receptor agonists [D-penicillamine(2,5)]-enkephalin. OATP8 and OATP-A also transport the opioid receptor agonist deltorphin (*Gastroenterology 120:525; J Pharmacol Exp Ther 294(1):73*).

[0044] 38555 is a critical gene in pain because it transports Prostaglandin E2 and may additionally transport several other molecules that are known to be involved in pain transmission. Furthermore, 38555 is localized in nociceptive neurons of the DRG. Blockers of 38555 would therefore be novel mechanisms for pain inhibition. Due to the expression of 38555 in the spinal cord and dorsal root ganglion, along with its functional role, modulators of 38555 activity would be useful in treating disorders associated with the treatment of pain and painful disorders. 38555 polypeptides of the present invention are useful in screening for modulators of 38555 activity.

Gene ID 55063

[0045] The human 55063 sequence (SEQ ID NO:21), known also as NMDA receptor subunit 3A (NR3A) or (NMDAR-L), is approximately 4197 nucleotides long

including untranslated regions. The coding sequence, located at about nucleic acid 1 to 3348 of SEQ ID NO:21, encodes a 1115 amino acid protein (SEQ ID NO:22).

[0046] As assessed by TaqMan analysis, 55063 mRNA was expressed predominantly in tissues of the central nervous system (CNS) in human and rat panels. 55063 mRNA was also down-regulated in the dorsal root ganglion (DRG) after Axotomy, Rhizotomy, Capsaicin, Spared Nerve Injury (SNI) and Tibial Nerve Injury (TNI) pain models. *In situ* hybridization experiments indicated that 55063 mRNA was restricted to a sub-population of neurons in the spinal cord, laminae and a sub-population of neurons in the cortex.

[0047] 55063 is NR3A (a.k.a. NMDAR-L). This NMDA receptor subunit is not functional by itself, but forms functional subunits when co-expressed with either the NR1 or NR2 subunits (*J Neurosci 15(10):6498; J Neurosci 15(10):6509; J Neurosci 21(4):1128*). NMDA receptors are well known mediators of pain transmission. In addition, many of the downstream effects of NMDA receptor are thought to be responsibe for NMDA receptor mediated pain transmission (for recent reviews, see *Ann N Y Acad Sci. 933:142-56; Curr Pharm Des. 8(10):887-912; Z Rheumatol. 60(6):404-15; Trends Pharmacol Sci. 22(12):636-42*). However, NMDA receptor blockers are well known to have a number of negative side effects due to their primary role in the central nervous system. Therefore, a drug which could increase the function of NR3A could decrease the NMDA receptor-mediated pain transmission by decreasing NMDA function and by decreasing calcium influx via NMDA receptors. In addition, since NR3A does not abolish NMDA receptor activity, side effects could be less than those caused by NMDA receptor antagonists.

[0048] 55063 plays a critical role in pain because it is localized to a subpopulation of neurons in the regions of the spinal cord where nociceptive neurons terminate and because it is down-regulated in the DRG of several models of pain. Furthermore, 55063 is known to decrease NMDA receptor mediated currents and calcium influx. Since NMDA receptor function and calcium influx are clearly linked to pain, a decrease in 55063 expression would therefore increase pain transmission. Therefore, drugs which increase 55063 function would be novel mechanisms to decrease pain transmission without the side effects commonly seen with antagonists which completely abolish NMDA receptor function. Due to the expression of 55063 in the spinal cord and dorsal root ganglion, along with its functional role, modulators of 55063 activity would be useful in treating disorders associated with the

treatment of pain and painful disorders. 55063 polypeptides of the present invention are useful in screening for modulators of 55063 activity.

Gene ID 57145

[0049] The human 57145 sequence (SEQ ID NO:23), known also Organic cation transporter 5 (OCT5), is approximately 2561 nucleotides long including untranslated regions. The coding sequence, located at about nucleic acid 184 to 1830 of SEQ ID NO:23, encodes a 548 amino acid protein (SEQ ID NO:24).

[0050] As assessed by TaqMan analysis, 57145 mRNA was primarily expressed in tissues of the central nervous system in human and rat panels. 57145 mRNA was up-regulated in the dorsal root ganglion (DRG) after Chronic Constriction Injury (CCI) and Complete Freund's Adjuvant (CFA). 57145 mRNA was also down-regulated in DRG and spinal cord after Rhizotomy and in spinal cord after CCI, CFA, and Axotomy. *In situ* hybridization experiments performed on 57145 mRNA showed diffuse expression in monkey brain, spinal cord and DRG neurons. It is also expressed in TRG, SCG neurons.

[0051] 57145 is a novel member of the organic cation family. Organic transporter activity is increased by PKC, Calmodulin (CaM), Protein Kinase A and CaM kinase II (J Am Soc Nephrol 11:1216; Am J Physiol Renal Physiol 284:F293) which are known to be critical mediators of pain transmission. (for recent review, see Eur J Pharmacol 429:23-37; Ann N Y Acad Sci 933:142-56). Furthermore, CCI and formalin injection models of pain cause a movement of PKC from the cytosol to the membrane in spinal cord nociceptive neurons, an effect known to be associated with an increase in PKC activity. This PKC translocation correlates well with an increase in hyperalgesia (*J Neurophysiol 70:470; Neurosci Lett 198:75; Pain 94:17).* In addition, PKC activation mediates the increase in NMDA receptor activity in the CFA model of pain, since PKC inhibitors block this NMDA receptor increase (*J Physiol 537:115*). Since CCI and CFA models of pain increase PKC levels which increase organic cation transport activity, small molecule inhibitors of 57145 may be a novel mechanism for the inhibition of pain.

[0052] 57145 plays an important role in pain transmission because it is up-regulated in DRG after CCI and CFA models of pain. Since CCI and CFA increase the activity of PKC, (known to be an important pain mediator) which in turn increases organic cation transport activity, small molecule inhibitors of 57145 could be a novel mechanism for the inhibition of pain. Due to the expression of 57145 in the spinal cord and dorsal root

ganglion, along with its functional role, modulators of 57145 activity would be useful in treating disorders associated with the treatment of pain and painful disorders. 57145 polypeptides of the present invention are useful in screening for modulators of 57145 activity.

Gene ID 59914

[0053] The human 59914 sequence (SEQ ID NO:25), known also as Choline transporter-like 3 (CTL3), is approximately 2500 nucleotides long including untranslated regions. The coding sequence, located at about nucleic acid 105 to 2258 of SEQ ID NO:25, encodes a 717 amino acid protein (SEQ ID NO:26).

[0054] As assessed by TaqMan analysis, 59914 mRNA was primarily expressed in human brain, muscle and spinal cord samples. *In situ* hybrization experiments indicated that 59914 mRNA was expressed in both human and rat brain, spinal cord and majority of dorsal root ganglion (DRG) neurons.

[0055] Many studies have demonstrated that cholinergic receptor (AChR) agonists inhibit pain whereas antagonists increase pain (for review, see *Annu Rev Physiol 59:457-82*). However, a recent study has demonstrated that mecamylamine, a non-subtype selective nAChR antagonist, can produce an inhibition of pain after formalin injection at early times, and an increase in pain at late times, suggesting that nAChRs may exert opposing effects on acute versus tonic pain (*Brain Res 888(1):102-106*). 59914 is a novel choline transporter that may serve to transport choline back into cholinergic nerve terminals following acetylcholine release and breakdown by acetylcholinesterase. Thus, inhibition of 59914 may serve to deplete DRG neurons of acetylcholine, thus inhibiting acute forms of pain transmission.

[0056] 59914 plays an important role in pain because it is localized in DRG and spinal cord neurons. Inhibition of 59914 potentially prevents the re-uptake of choline into cholinergic neurons, thereby depleting cholinergic cells of acetylcholine. Because of the importance of acetylcholine in pain transmission, such 59914 inhibitors may be a novel mechanism for the inhibition of acute pain. Due to the expression of 59914 in the spinal cord, along with its functional role, modulators of 59914 activity would be useful in treating disorders associated with the treatment of pain and painful disorders. 59914 polypeptides of the present invention are useful in screening for modulators of 59914 activity.

Gene ID 94921

[0057] The human 94921 sequence (SEQ ID NO:27), known also as Equilibrative nucleoside transporter 4 (ENT4), is approximately 2763 nucleotides long including untranslated regions. The coding sequence, located at about nucleic acid 34 to 1626 of SEQ ID NO:27, encodes a 530 amino acid protein (SEQ ID NO:28).

[0058] As assessed by TaqMan analysis, 94921 mRNA was primarily expressed in the human brain, pancreas, spinal cord and in rat nervous system tissue samples. 94921 mRNA was up-regulated in dorsal root ganglion (DRG) after Chronic constriction Injury (CCI) and Axotomy. *In situ* hybridization experiments indicated that 94921 mRNA was expressed in mouse brain, spinal cord and in a subpopulation (small and medium size) of the DRG neurons.

[0059] 94921 is a novel member of the equilibrative nucleoside transporter (ENT) family, which transports both purines and pyrimidines. Since this family is equilibrative, it can take up or release nucleosides, depending on the concentration gradient. Extracellular purines stimulate the synthesis and release of trophic factors such as nerve growth factor (NGF), S100beta protein and transforming growth factor beta from astrocytes (for review, see *Int J Dev Neurosci 19(4):395-414*). Many studies indicate that growth factors such as NGF are mediators of neuropathic pain such as that produced in chronic constriction injury (CCI) models (for review, see *Curr Opin Pharmacol 1(1):66-72*). The levels of both NGF and 94921 increase in DRG after CCI, suggest that 94921 may be a primary cause of the increase in NGF. Therefore, blockade of 94921 could prevent the increase in NGF levels following CCI and could therefore be a novel mechanism for the inhibition of pain associated with neuropathy.

[0060] 94921 plays an important role in pain transmission during painful neuropathy, because levels of 94921 and NGF increase in DRG after CCI. Since 94921 transports adenosine, a known player in pain transmission, drugs which inhibit 94921 could be a novel way of inhibiting NGF levels after neuropathy, and could therefore be a novel mechanism for the inhibition of pain. Due to the expression of 94921 in the spinal cord and dorsal root ganglion, along with its functional role, modulators of 94921 activity would be useful in treating disorders associated with the treatment of pain and painful disorders.

94921 polypeptides of the present invention are useful in screening for modulators of 94921 activity.

Gene ID 16852

[0061] The human 16852 sequence (SEQ ID NO:29), known also as large-conductance calcium-activated potassium channel (*slowpoke*) beta subunit KCNMB4, is approximately 1107 nucleotides long including untranslated regions. The coding sequence, located at about nucleic acid 56 to 688 of SEQ ID NO:29, encodes a 210 amino acid protein (SEQ ID NO:30).

[0062] As assessed by TaqMan analysis, 16852 mRNA was primarily expressed in tissues of the central nervous system in human and rat panels. 16852 mRNA was up-regulated in the dorsal root ganglion (DRG) after Axotomy, Chronic Constriction Injury (CCI) and Spared Nerve Injury (SNI) and in the in spinal cord after CCI and Axotomy. *In situ* hybridization experiments indicated that 16852 mRNA was expressed in both human, monkey and rat brains and in monkey spinal cord and small and medium size of the DRG neurons.

[0063] Activation of potassium channels affects the frequency and the pattern of neuronal firing. Several voltage-gated potassium channels are expressed in sub-population of sensory neurons including those involved in nociception. In general, it has been shown that the expression of some voltage-gated potassium channels decreases in DRG neurons after axotomy and that the peak of potassium currents is reduced in sensory neurons during chronic inflammation. Furthermore, administration of potassium channel openers potentiated the antinociception produced by agonists of alpha-2-adrenoreceptors or by morphine. The antinociception induced by intrathecal injection of morphine.injection is blocked in a dose-dependent manner by glibenclamide (a blocker of ATP-sensitive K+ channels) and potentiated by nicorandil (a opener of ATP-sensitive K+ channels).

[0064] 16852 is a b subunit of the large conductance, calcium-dependent K+ channel, Slowpoke. When co-expressed with Slowpoke, 16852 down-regulates Slowpoke channel activity by shifting its activation range to more depolarized voltages and slowing its activation kinetics. 16852 may play an important role in pain transmission because it is up-regulated in several pain models. Inhibitors of 16852 will increase large conductance, calcium-dependent potassium channel activity and thus decrease neuronal firing. Thus, drugs which inhibit 16852 may be novel methods for the inhibition of pain. Due to the

expression of 16852 in the spinal cord and dorsal root ganglion, along with its functional role, modulators of 16852 activity would be useful in treating disorders associated with the treatment of pain and painful disorders. 16852 polypeptides of the present invention are useful in screening for modulators of 16852 activity.

Gene ID 33260

[0065] The human 33260 sequence (SEQ ID NO:31), known also as Potassium voltage-gated channel subfamily H member 1 (KCNH1); or as Ether-a-go-go potassium channel 1 (hEAG1), is approximately 3083 nucleotides long including untranslated regions. The coding sequence, located at about nucleic acid 37 to 3006 of SEQ ID NO:31, encodes a 989 amino acid protein (SEQ ID NO:32).

[0066] As assessed by TaqMan analysis, 33260 mRNA was primarily expressed in tissues of the central nervous system (CNS) in both monkey and rat panels. 33260 mRNA was up-regulated in the dorsal root ganglion (DRG) after Chronic Constriction Injury (CCI) and in the spinal cord after Capsaicin treatment. *In situ* hybridization experiments indicated that 33260 mRNA was expressed in a sub-population of DRG neurons in monkey and rat and in the spinal cord, with highest levels in the superficial laminae of the dorsal horn, in both monkey and rat. 33260 was also shown to be expressed in a sub-population of cortical neurons in both monkey and rat.

[0067] Activation of potassium channels affects the frequency and the pattern of neuronal firing. Several voltage-gated potassium channels are expressed in sub-population of sensory neurons including those involved in nociception. In general, it has been shown that the expression of some voltage-gated potassium channels decreases in DRG neurons after axotomy and that the peak of potassium currents is reduced in sensory neurons during chronic inflammation. Furthermore, administration of potassium channel openers potentiated the antinociception produced by agonists of alpha-2-adrenoreceptors or by morphine. The antinociception induced by intrathecal injection of morphine.injection is blocked in a dose-dependent manner by glibenclamide (a blocker of ATP-sensitive K+ channels) and potentiated by nicorandil (a opener of ATP-sensitive K+ channels).

[0068] 33260 (the potassium channel EAG-1) is important for the modulation of the firing pattern of nociceptive neurons. Drugs which open 33260 may therefore be a novel mechanism for the inhibition of pain. Due to the expression of 33260 in the spinal cord and dorsal root ganglion, along with its functional role, modulators of 33260 activity would be

useful in treating disorders associated with the treatment of pain and painful disorders. 33260 polypeptides of the present invention are useful in screening for modulators of 33260 activity.

Gene ID 58573

[0069] The human 58573 sequence (SEQ ID NO:33), known also as a sodium-bicarbonate co-transporter NBC4, is approximately 6313 nucleotides long including untranslated regions. The coding sequence, located at about nucleic acid 348 to 3713 of SEQ ID NO:33, encodes a 1121 amino acid protein (SEQ ID NO:34).

[0070] As assessed by TaqMan analysis, 58573 mRNA was expressed in tissues of the central nervous system (CNS) and in kidney tissue samples in both monkey and rat panels. 58573 mRNA was up-regulated in the dorsal root ganglion (DRG) after Chronic Constriction Injury (CCI) and in C5-T1 DRG 4 weeks post-Rhizotomy. 58573 mRNA was up-regulated in spinal cord after Axotomy. 58573 mRNA was down-regulated in DRG after Complete Freund's Adjuvant (CFA) and in C3 DRG 4 weeks post-Rhizotomy. 58573 mRNA was down-regulated in the DRG and spinal cord after Spared Nerve Injury (SNI), Tibial Nerve Injury (TNI) and long-term Capsaicin treatment. *In situ* hybridization experiments indicated that 58573 mRNA was expressed in a sub-population of small neurons in the DRG and in a sub-population of thalamic neurons in the monkey.

[0071] 58573 is an important gene in the sensation of pain because it is present in nociceptive neurons of the DRG and in sensory thalamic neurons and it is up-regulated in DRG and spinal cord in some models of pain. 58573 is a member of the sodium-bicarbonate cotransporter (NBC) family. NBC's are known to transport bicarbonate into cells and are thought to be at least partially responsible for extracellular acidification after neuronal activity. Since protons are known mediators of pain, inhibition of 58573 will be a novel mechanism for the inhibition of pain. Due to the expression of 58573 in the spinal cord and dorsal root ganglion, along with its functional role, modulators of 58573 activity would be useful in treating disorders associated with the treatment of pain and painful disorders. 58573 polypeptides of the present invention are useful in screening for modulators of 58573 activity.

Gene ID 30911

[0072] The human 30911 sequence (SEQ ID NO:35), known also a sodium-proton exchanger (NHE5), is approximately 3761 nucleotides long including untranslated regions. The coding sequence, located at about nucleic acid 70 to 2760 of SEQ ID NO:35, encodes a 896 amino acid protein (SEQ ID NO:36).

[0073] As assessed by TaqMan analysis, 30911 mRNA was expressed at highest levels in tissues of the central nervous system (CNS) of human and rat models. 30911 mRNA was up-regulated at multiple time points in dorsal root ganglion (DRG) after Chronic Constriction Injury (CCI). 30911 mRNA was down-regulated in early time points in the DRG after Spared Nerve Injury (SNI) and after long-term Capsaicin treatment.

[0074] 30911 is an important gene in the sensation of pain because it is up-regulated in DRG in a neuropathic model of pain. 30911 is a member of the sodium-proton exchanger (NHE) family. NHE play an important role in pH regulation, since they pump protons out of the cell. Since protons are known mediators of pain, inhibiting 30911 will be a novel mechanism for the inhibition of pain. Due to 30911 mRNA expression in the dorsal root ganglion, along with its functional role, modulators of 30911 activity would be useful in treating disorders associated with the treatment of pain and painful disorders. 30911 polypeptides of the present invention are useful in screening for modulators of 30911 activity.

Gene ID 85913

[0075] The human 85913 sequence (SEQ ID NO:37), a member of the hyperpolarization activated cyclic nucleotide-gated potassium channel family (HCN1), is approximately 4069 nucleotides long including untranslated regions. The coding sequence, located at about nucleic acid 43 to 2691 of SEQ ID NO:37, encodes a 882 amino acid protein (SEQ ID NO:38).

[0076] As assessed by TaqMan analysis, 85913 mRNA was expressed at highest levels in tissues of the central nervous system (CNS) of human and rat models. 85913 was also shown to be highly expressed in rat bladder tissue samples. Additionally, 85913 was shown to be up-regulated in dorsal root ganglion (DRG) samples after Axotomy, Tibial Nerve Injury (TNI) and Spared Nerve Injury (SNI). 85913 was also shown to be down-regulated in spinal cord samples after Axotomy, Complete Freund's Adjuvant (CFA), SNI and Rhizotomy. 85913 was additionally shown to be down-regulated in DRG after chronic constriction injury (CCI), CFA and Rhizotomy. No regulation was observed in DRG and

spinal cord samples after capsaicin treatment. *In situ* hybridization experiments demonstrated that 85913 was expressed in both human and rat brains, dorsal horn or the spinal cord and subpopulations of the DRG neurons.

[0077] 85913, known also as HCN1, is a member of the hyperpolarization activated cyclic nucleotide-gated potassium channel family. HCN channels are permeate to both Na+ and K + and open upon hyperpolarization. Since they depolarize the cell at negative potentials, they act as "pacemaker" channels, providing a mechanism for increasing the action potential firing rate (for review, see *News Physiol Sci 17:32-7*). Since 85913 is localized in the dorsal horn of the spinal cord and in a subpopulation of DRG neurons, and since 85913 may be important for increasing the action potential rate from nociceptive neurons, 85913 may play a critical role in the transmission of pain information. Therefore, without being bound by theory, inhibiting 85913 may be a novel mechanism for the inhibition of pain. Due to 85913 mRNA expression in the dorsal root ganglion, along with its functional role, modulators of 85913 activity would be useful in treating disorders associated with the treatment of pain and painful disorders. 85913 polypeptides of the present invention are useful in screening for modulators of 85913 activity.

Gene ID 14303

[0078] The human 14303 sequence (SEQ ID NO:39), a sodium-proton exchanger (NHE6), is approximately 4452 nucleotides long including untranslated regions. The coding sequence, located at about nucleic acid 36 to 2045 of SEQ ID NO:39, encodes a 669 amino acid protein (SEQ ID NO:40)

[0079] As assessed by TaqMan analysis, 14303 mRNA was expressed at highest levels in tissues of the central nervous system (CNS) of human and rat models. Additionally, 14303 was shown to be up-regulated in dorsal root ganglion (DRG) samples after chronic constriction injury (CCI). 14303 was also shown to be down-regulated in DRG and spinal cord samples after Tibial Nerve Injury (TNI) and it was shown to be down-regulated in DRG after Spared Nerve Injury (SNI). 14303 was additionally shown to be down-regulated in C3 DRG after Rhizotomy and it was shown to be down-regulated in long term DRG after capsaicin treatment.

[0080] *In situ* hybridization experiments demonstrated that 14303 was expressed in all neurons of DRG, spinal cord and cortex. 14303 was also demonstrated to be expressed in monkey prostate muscle cells.

[0081] 14303 is also known as a sodium-proton exchanger (NHEs), which are thought to be important regulators of pH in and around neurons by pumping protons out of cells following neuronal activity (see, for example, *J Clin Invest 104(5):637-45; J Neurosci Res 56(4):358-70; J Neurosci Res 1998 Feb 15;51(4):431-41*). Extracellular acidification has been shown to mediate pain. Continuous administration of low pH solutions in humans causes pain and hyperalgesia (*Neurosci Lett* 154:113-116). In addition, protons selectively induce lasting excitation and sensitization to mechanical stimulation of nociceptors in rat skin, in vitro (*J Neurosci* 12(1):86-95). Most importantly, protons directly activate VR-1 receptors present in nociceptive neurons in the DRG (*J Physiol* 433:145-61; *TIPS* 20:(3):112-8). VR-1 receptors are important players in pathways involved in pain. Therefore, 14303 may be an important gene in the sensation of pain because it is up-regulated in DRG in a neuropathic model of pain. 14303 is a member of the sodium-proton exchanger (NHE) family. NHE's are thought to play an important role in pH regulation, since they pump protons out of the cell. Since protons are known mediators of pain, inhibition of 14303 may be a novel mechanism for the inhibition of pain. Due to 14303 mRNA expression in the dorsal root ganglion, along with its functional role, modulators of 14303 activity would be useful in treating disorders associated with the treatment of pain and painful disorders. 14303 polypeptides of the present invention are useful in screening for modulators of 14303 activity.

Gene ID 16816

[0082] The human 16816 sequence (SEQ ID NO:41), a Phospholipase C Delta 4, is approximately 3116 nucleotides long including untranslated regions. The coding sequence, located at about nucleic acid 205 to 2493 of SEQ ID NO:41, encodes a 762 amino acid protein (SEQ ID NO:42)

[0083] As assessed by TaqMan analysis using a human tissue panel, 16816 mRNA was expressed at highest levels in skeletal muscle tissues, followed by bladder tissue samples and tissues of the central nervous system (CNS). Additional TaqMan experiments on a rat tissue panel demonstrated expression in skeletal muscle, bladder and CNS tissue samples. Additionally, 16816 was shown to be up-regulated as well as down-regulated in dorsal root ganglion (DRG) samples after chronic constriction injury (CCI). 16816 was also shown to be down-regulated in DRG and up-regulated in spinal cord samples after Spared Nerve Injury (SNI).

[0084] *In situ* hybridization experiments demonstrated that 16816 was expressed in nociceptive neurons in both monkey and rat DRG samples. 16816 was also demonstrated to be expressed in monkey brain (thalamus) and muscle tissue samples.

[0085] 16816 is also known as a phatidylinositol-specific phospholipase C (PLC), which plays an important role in receptor-mediated signal transduction by generating 2 second messenger molecules, inositol 1,4,5-triphosphate (IP3) and diacylglycerol, from phosphatidylinositol 4,5-bisphosphate (PIP2). PLC is well known to be involved in nociceptive pathways. It is well established, for example, that bradykinin and nerve growth factor (NGF), two pro-algesic agents, activate G-protein-coupled (BK2) and tyrosine kinase (TrkA) receptors, respectively, to stimulate phospholipase C (PLC) signalling pathways in primary afferent neurons. It has been shown that bradykinin- or NGF-mediated potentiation of thermal sensitivity in vivo requires expression of VR1, a well established receptor involved in pain. Diminution of plasma membrane phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P2) levels through antibody sequestration or PLC-mediated hydrolysis mimics the potentiating effects of bradykinin or NGF at the cellular level. Moreover, recruitment of PLC-gamma to TrkA is essential for NGF-mediated potentiation of channel activity, and biochemical studies suggest that VR1 associates with this complex (*Nature*. 2001 411(6840):957-62). Given the importance of PLC in pain pathways, drugs which modulate 16816 may be important for the treatment of pain. Due to 16816 mRNA expression in the dorsal root ganglion, along with its functional role, modulators of 16816 activity would be useful in treating disorders associated with the treatment of pain and painful disorders. 16816 polypeptides of the present invention are useful in screening for modulators of 16816 activity.

Gene ID 17827

[0086] The human 17827 sequence (SEQ ID NO:43), a potassium channel KCNK12 (THIK2), is approximately 1943 nucleotides long including untranslated regions. The coding sequence, located at about nucleic acid 466 to 1758 of SEQ ID NO:43, encodes a 430 amino acid protein (SEQ ID NO:44)

[0087] As assessed by TaqMan analysis, 17827 mRNA was expressed at highest levels in tissues of the central nervous system (CNS) of human and rat models. Expression of 17827 was additionally observed in lung, prostate and trachea tissue samples in the rat panel. Additionally, 17827 was shown to be up-regulated in dorsal root ganglion (DRG)

samples after chronic constriction injury (CCI). 17827 was also shown to be up-regulated in spinal cord samples after Spared Nerve Injury (SNI). 17827 was additionally shown to be down-regulated in L5, L6 DRG samples after Spinal Nerve Ligation (SNL) after 84 days.

[0088] *In situ* hybridization experiments demonstrated that 17827 was expressed in both monkey and rat DRG neurons of all sizes, TRG. 17827 was also demonstrated to be expressed in monkey and rat spinal cord and brain samples.

[0089] 17827 is a potassium channel present in DRG neurons. Activation of potassium channels affect the frequency and the pattern of neuronal firing. Modulation of potassium channels may be important for the firing pattern of nociceptive neurons. Therefore drugs which interact with 17827 could be a novel mechanism for the inhibition of pain. Due to 17827 mRNA expression in the dorsal root ganglion and spinal cord, along with its functional role, modulators of 17827 activity would be useful in treating disorders associated with the treatment of pain and painful disorders. 17827 polypeptides of the present invention are useful in screening for modulators of 17827 activity.

Gene ID 32620

[0090] The human 32620 sequence (SEQ ID NO:45), a sodium-glucose cotransporter SMIT2, is approximately 2384 nucleotides long including untranslated regions. The coding sequence, located at about nucleic acid 233 to 2260 of SEQ ID NO:45, encodes a 675 amino acid protein (SEQ ID NO:46)

[0091] As assessed by TaqMan analysis, 32620 mRNA was expressed at highest levels in tissues of the central nervous system (CNS) of human and rat models. Expression of 32620 was additionally observed in colon and small intestine tissue samples in the human panel and in the kidney, prostate and duodenum samples in the rat panel. Additionally, 32620 was shown to be down-regulated in dorsal root ganglion (DRG) samples after chronic constriction injury (CCI). 32620 was also shown to be up-regulated in DRG and spinal cord samples after axotomy. 32620 was additionally shown to be down-regulated in DRG after Spared Nerve Injury (SNI).

[0092] *In situ* hybridization experiments demonstrated that 32620 was mainly expressed in small diameter neurons of DRG. 32620 was also shown to be expressed in glial cells of the spinal cord and cortex of monkey tissues.

[0093] 32620 transports myo-inositol in a sodium-dependent manner. Intrathecal injection of myo-inositol (2.5 mg) significantly reversed both the anti-hyperalgesic and anti-

allodynic effect of intrathecal injection of lithium (*Pain* 2000 85:59-64). Thus, drugs which interact with 32620 may be important for the treatment of pain. Due to 32620 mRNA expression in the dorsal root ganglion and spinal cord, along with its functional role, modulators of 32620 activity would be useful in treating disorders associated with the treatment of pain and painful disorders. 32620 polypeptides of the present invention are useful in screening for modulators of 32620 activity.

[0094] Various aspects of the invention are described in further detail in the following subsections:

Screening Assays:

[0095] The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules (organic or inorganic) or other drugs) which bind to 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 proteins, have a stimulatory or inhibitory effect on, for example, 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 expression or 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity, or have a stimulatory or inhibitory effect on, for example, the expression or activity of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 substrate. Compounds identified using the assays described herein may be useful for treating pain and painful conditions.

[0096] These assays are designed to identify compounds that bind to a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein, bind to other intracellular or extracellular proteins that interact with a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein, and interfere with the interaction of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555,

55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein with other intercellular or extracellular proteins. For example, in the case of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein, which is a transmembrane receptor-type protein, such techniques can identify ligands for such a receptor. A 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein ligand or substrate can, for example, be used to ameliorate pain and painful conditions. Such compounds may include, but are not limited to peptides, antibodies, or small organic or inorganic compounds. Such compounds may also include other cellular proteins.

[0097] Compounds identified via assays such as those described herein may be useful, for example, for treating pain and painful conditions. In instances whereby a painful condition results from an overall lower level of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene expression and/or 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein in a cell or tissue, compounds that interact with the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein may include compounds which accentuate or amplify the activity of the bound 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein. Such compounds would bring about an effective increase in the level of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein activity, thus ameliorating symptoms.

[0098] In other instances, mutations within the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene may cause aberrant types or excessive amounts of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 proteins to be made which have a deleterious effect that leads to a pain. Similarly,

physiological conditions may cause an excessive increase in 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene expression leading pain. In such cases, compounds that bind to a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein may be identified that inhibit the activity of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein. Assays for testing the effectiveness of compounds identified by techniques such as those described in this section are discussed herein.

[0099] In one embodiment, the invention provides assays for screening candidate or test compounds which are substrates of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein or polypeptide or biologically active portion thereof. In another embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein or polypeptide or biologically active portion thereof. The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. (1997) *Anticancer Drug Des.* 12:145).

[00100] Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.* (1994). *J. Med. Chem.* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in Gallop *et al.* (1994) *J. Med. Chem.* 37:1233.

[00101] Libraries of compounds may be presented in solution (*e.g.*, Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner USP 5,223,409), spores (Ladner USP '409), plasmids (Cull *et al.* (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and Smith (1990) *Science* 249:386-390); (Devlin (1990) *Science* 249:404-406); (Cwirla *et al.* (1990) *Proc. Natl. Acad. Sci.* 87:6378-6382); (Felici (1991) *J. Mol. Biol.* 222:301-310); (Ladner *supra.*).

[00102] In one embodiment, an assay is a cell-based assay in which a cell which expresses a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity is determined. Determining the ability of the test compound to modulate 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity can be accomplished by monitoring, for example, intracellular calcium, IP_3, cAMP, or diacylglycerol concentration, the phosphorylation profile of intracellular proteins, cell proliferation and/or migration, gene expression of, for example, cell surface adhesion molecules or genes associated with analgesia, or the activity of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 - regulated transcription factor. The cell can be of mammalian origin, *e.g.*, a neural cell. In one embodiment, compounds that interact with a receptor domain can be screened for their ability to function as ligands, *i.e.*, to bind to the receptor and modulate a signal transduction pathway. Identification of ligands, and measuring the activity of the ligand-receptor complex, leads to the identification of modulators (*e.g.*, antagonists) of this interaction. Such modulators may be useful in the treatment of pain and painful conditions.

[00103] The ability of the test compound to modulate 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 binding to a substrate or to bind to 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 can also

be determined. Determining the ability of the test compound to modulate 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 binding to a substrate can be accomplished, for example, by coupling the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 substrate with a radioisotope or enzymatic label such that binding of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 substrate to 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 can be determined by detecting the labeled 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 substrate in a complex. 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 could also be coupled with a radioisotope or enzymatic label to monitor the ability of a test compound to modulate 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 binding to a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 substrate in a complex. Determining the ability of the test compound to bind 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 can be accomplished, for example, by coupling the compound with a radioisotope or enzymatic label such that binding of the compound to 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 can be determined by detecting the labeled 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 compound in a complex. For example, compounds (*e.g.*, 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 ligands or substrates) can be labeled with ^{125}I, ^{35}S, ^{14}C, or ^{3}H, either directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by

scintillation counting. Compounds can further be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

[00104] It is also within the scope of this invention to determine the ability of a compound (*e.g.*, a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 ligand or substrate) to interact with 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 without the labeling of any of the interactants. For example, a microphysiometer can be used to detect the interaction of a compound with 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 without the labeling of either the compound or the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 (McConnell, H. M. *et al.* (1992) *Science* 257:1906-1912. As used herein, a "microphysiometer" (*e.g.*, Cytosensor) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a compound and 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620.

[00105] In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 target molecule (*e.g.*, a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 substrate) with a test compound and determining the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 target molecule. Determining the ability of the test compound to modulate the activity of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 target molecule can be

accomplished, for example, by determining the ability of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein to bind to or interact with the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 target molecule.

[00106] Determining the ability of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein or a biologically active fragment thereof, to bind to or interact with a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 target molecule can be accomplished by one of the methods described above for determining direct binding. In a preferred embodiment, determining the ability of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein to bind to or interact with a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (*i.e.*, intracellular Ca^{2+}, diacylglycerol, IP_3, cAMP), detecting catalytic/enzymatic activity of the target on an appropriate substrate, detecting the induction of a reporter gene (comprising a target-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a target-regulated cellular response (*e.g.*, gene expression).

[00107] In yet another embodiment, an assay of the present invention is a cell-free assay in which a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein or biologically active portion thereof, is contacted with a test compound and the ability of the test compound to bind to the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein or biologically active portion thereof is determined. Preferred biologically active portions of the 16386, 15402, 21165, 1423, 636,

12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 proteins to be used in assays of the present invention include fragments which participate in interactions with non-16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 molecules, *e.g.*, fragments with high surface probability scores. Binding of the test compound to the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein can be determined either directly or indirectly as described above. In a preferred embodiment, the assay includes contacting the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein or biologically active portion thereof with a known compound which binds 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein, wherein determining the ability of the test compound to interact with a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein comprises determining the ability of the test compound to preferentially bind to 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 or biologically active portion thereof as compared to the known compound. Compounds that modulate the interaction of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 with a known target protein may be useful in regulating the activity of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein, especially a mutant 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein.

[00108] In another embodiment, the assay is a cell-free assay in which a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein or biologically active portion thereof is determined. Determining the ability of the test compound to modulate the activity of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein can be accomplished, for example, by determining the ability of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein to bind to a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 target molecule by one of the methods described above for determining direct binding. Determining the ability of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein to bind to a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 target molecule can also be accomplished using a technology such as real-time Biomolecular Interaction Analysis (BIA) (Sjolander, S. and Urbaniczky, C. (1991) *Anal. Chem.* 63:2338-2345 and Szabo *et al.* (1995) *Curr. Opin. Struct. Biol.* 5:699-705). As used herein, "BIA" is a technology for studying biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore). Changes in the optical phenomenon of surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

[00109] In another embodiment, determining the ability of the test compound to modulate the activity of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein can be accomplished by determining the ability of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein to further

modulate the activity of a downstream effector of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 target molecule. For example, the activity of the effector molecule on an appropriate target can be determined or the binding of the effector to an appropriate target can be determined as previously described.

[00110] In yet another embodiment, the cell-free assay involves contacting a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein or biologically active portion thereof with a known compound which binds the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein, wherein determining the ability of the test compound to interact with the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein comprises determining the ability of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein to preferentially bind to or modulate the activity of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 target molecule.

[00111] In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein, or interaction of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein

with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtitre plates, which are then combined with the test compound or the test compound and either the non-adsorbed target protein or 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein, and the mixture incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtitre plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 binding or activity determined using standard techniques.

[00112] Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein or a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554,

38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein or target molecules but which do not interfere with binding of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein to its target molecule can be derivatized to the wells of the plate, and unbound target or 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein or target molecule.

[00113] In another embodiment, modulators of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 mRNA or protein in the cell is determined. The level of expression of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 mRNA or protein in the presence of the candidate compound is compared to the level of expression of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 expression based on this comparison. For example, when expression of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911,

85913, 14303, 16816, 17827 or 32620 mRNA or protein is greater (statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 mRNA or protein expression. Alternatively, when expression of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 mRNA or protein expression. The level of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 mRNA or protein expression in the cells can be determined by methods described herein for detecting 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 mRNA or protein.

[00114] In yet another aspect of the invention, the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 proteins can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, *e.g.*, U.S. Patent No. 5,283,317; Zervos *et al.* (1993) *Cell* 72:223-232; Madura *et al.* (1993) *J. Biol. Chem.* 268:12046-12054; Bartel *et al.* (1993) *Biotechniques* 14:920-924; Iwabuchi *et al.* (1993) *Oncogene* 8:1693-1696; and Brent WO94/10300), to identify other proteins, which bind to or interact with 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 ("16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 -binding proteins" or "16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 -bp") and are involved in 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity. Such 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555,

55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 -binding proteins are also likely to be involved in the propagation of signals by the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 proteins or 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 targets as, for example, downstream elements of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 -mediated signaling pathway. Alternatively, such 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 -binding proteins are likely to be 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 inhibitors.

[00115] The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo,* forming a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 -dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene which encodes the protein which interacts with the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein.

[00116] In another aspect, the invention pertains to a combination of two or more of the assays described herein. For example, a modulating agent can be identified using a cell-based or a cell free assay, and the ability of the agent to modulate the activity of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein can be confirmed *in vivo*, *e.g.*, in an animal such as an animal model for pain, as described herein.

[00117] This invention further pertains to novel agents identified by the above-described screening assays. Accordingly, it is within the scope of this invention to further use an agent identified as described herein in an appropriate animal model. For example, an agent identified as described herein (*e.g.*, a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 modulating agent, an antisense 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 nucleic acid molecule, a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 -specific antibody, or a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 -binding partner) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

[00118] Any of the compounds, including but not limited to compounds such as those identified in the foregoing assay systems, may be tested for the ability to ameliorate pain. Cell-based and animal model-based assays for the identification of compounds exhibiting such an ability to ameliorate pain are described herein.

[00119] In addition, animal-based models of pain, such as those described herein, may be used to identify compounds capable of treating pain and painful conditions. Such animal models may be used as test substrates for the identification of drugs, pharmaceuticals, therapies, and interventions which may be effective in treating pain. For example, animal models may be exposed to a compound, suspected of exhibiting an ability to treat pain, at a sufficient concentration and for a time sufficient to elicit such an

amelioration of pain in the exposed animals. The response of the animals to the exposure may be monitored by assessing the reversal of the symptoms of pain before and after treatment.

[00120] With regard to intervention, any treatments which reverse any aspect of pain (i.e. have an analgesic effect) should be considered as candidates for human pain therapeutic intervention. Dosages of test agents may be determined by deriving dose-response curves.

[00121] Additionally, gene expression patterns may be utilized to assess the ability of a compound to ameliorate pain. For example, the expression pattern of one or more genes may form part of a "gene expression profile" or "transcriptional profile" which may be then be used in such an assessment. "Gene expression profile" or "transcriptional profile", as used herein, includes the pattern of mRNA expression obtained for a given tissue or cell type under a given set of conditions. Gene expression profiles may be generated, for example, by utilizing a differential display procedure, Northern analysis and/or RT-PCR. In one embodiment, 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene sequences may be used as probes and/or PCR primers for the generation and corroboration of such gene expression profiles.

[00122] Gene expression profiles may be characterized for known states, either pain or painful disorders or normal, within the cell- and/or animal-based model systems. Subsequently, these known gene expression profiles may be compared to ascertain the effect a test compound has to modify such gene expression profiles, and to cause the profile to more closely resemble that of a more desirable profile.

[00123] For example, administration of a compound may cause the gene expression profile of a pain disease model system to more closely resemble the control system. Administration of a compound may, alternatively, cause the gene expression profile of a control system to begin to mimic pain or a painful disease state. Such a compound may, for example, be used in further characterizing the compound of interest, or may be used in the generation of additional animal models.

Cell- and Animal-Based Model Systems

[00124] Described herein are cell- and animal-based systems which act as models for pain. These systems may be used in a variety of applications. For example, the cell- and animal-based model systems may be used to further characterize differentially expressed genes associated with pain or painful disorders, *e.g.*, 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620. In addition, animal- and cell-based assays may be used as part of screening strategies designed to identify compounds which are capable of ameliorating pain, as described, below. Thus, the animal- and cell-based models may be used to identify drugs, pharmaceuticals, therapies and interventions which may be effective in treating pain or painful disorders. Furthermore, such animal models may be used to determine the LD50 and the ED50 in animal subjects, and such data can be used to determine the *in vivo* efficacy of potential pain treatments.

Animal-Based Systems

[00125] Animal-based model systems of pain may include, but are not limited to, non-recombinant and engineered transgenic animals.

[00126] Non-recombinant animal models for pain may include, for example, genetic models.

[00127] Additionally, animal models exhibiting pain may be engineered by using, for example, 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene sequences described above, in conjunction with techniques for producing transgenic animals that are well known to those of skill in the art. For example, 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene sequences may be introduced into, and overexpressed in, the genome of the animal of interest, or, if endogenous 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene sequences are present, they may either be overexpressed or, alternatively, be disrupted in order to underexpress or inactivate 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene expression.

[00128] The host cells of the invention can also be used to produce non-human transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 -coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 sequences have been introduced into their genome or homologous recombinant animals in which endogenous 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 sequences have been altered. Such animals are useful for studying the function and/or activity of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 and for identifying and/or evaluating modulators of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, and the like. A transgene is exogenous DNA which is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

[00129] A transgenic animal used in the methods of the invention can be created by introducing a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 -

encoding nucleic acid into the male pronuclei of a fertilized oocyte, *e.g.*, by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 cDNA sequence can be introduced as a transgene into the genome of a non-human animal. Alternatively, a nonhuman homologue of a human 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene, such as a mouse or rat 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene, can be used as a transgene. Alternatively, a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene homologue, such as another 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 family member, can be isolated based on hybridization to the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 cDNA sequences and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 transgene to direct expression of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009, both by Leder *et al.*, U.S. Patent No. 4,873,191 by Wagner *et al.* and in Hogan, B., *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 transgene in its genome

and/or expression of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein can further be bred to other transgenic animals carrying other transgenes.

[00130] To create a homologous recombinant animal, a vector is prepared which contains at least a portion of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene. The 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene can be a human gene but more preferably, is a non-human homologue of a human 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene. For example, a rat 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene can be used to construct a homologous recombination nucleic acid molecule, *e.g.*, a vector, suitable for altering an endogenous 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene in the mouse genome. In a preferred embodiment, the homologous recombination nucleic acid molecule is designed such that, upon homologous recombination, the endogenous 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector). Alternatively, the homologous recombination nucleic acid molecule can be designed such that, upon homologous recombination, the endogenous 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921,

16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene is mutated or otherwise altered but still encodes functional protein (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein). In the homologous recombination nucleic acid molecule, the altered portion of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene is flanked at its 5' and 3' ends by additional nucleic acid sequence of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene to allow for homologous recombination to occur between the exogenous 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene carried by the homologous recombination nucleic acid molecule and an endogenous 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene in a cell, *e.g.*, an embryonic stem cell. The additional flanking 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 nucleic acid sequence is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the homologous recombination nucleic acid molecule (see, *e.g.*, Thomas, K.R. and Capecchi, M. R. (1987) *Cell* 51:503 for a description of homologous recombination vectors). The homologous recombination nucleic acid molecule is introduced into a cell, *e.g.*, an embryonic stem cell line (*e.g.*, by electroporation) and cells in which the introduced 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene has homologously recombined with the endogenous 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene are selected (see *e.g.*, Li, E. *et al.* (1992) *Cell* 69:915). The selected cells can then injected into a blastocyst of an animal (*e.g.*, a mouse) to form aggregation chimeras (see *e.g.*, Bradley, A. in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E.J. Robertson, ed. (IRL, Oxford, 1987) pp. 113-152). A

chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination nucleic acid molecules, *e.g.*, vectors, or homologous recombinant animals are described further in Bradley, A. (1991) *Current Opinion in Biotechnology* 2:823-829 and in PCT International Publication Nos.: WO 90/11354 by Le Mouellec *et al.*; WO 91/01140 by Smithies *et al.*; WO 92/0968 by Zijlstra *et al.*; and WO 93/04169 by Berns *et al.*

[00131] In another embodiment, transgenic non-human animals for use in the methods of the invention can be produced which contain selected systems which allow for regulated expression of the transgene. One example of such a system is the *cre/loxP* recombinase system of bacteriophage P1. For a description of the *cre/loxP* recombinase system, see, *e.g.*, Lakso *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman *et al.* (1991) *Science* 251:1351-1355. If a *cre/loxP* recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the *Cre* recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, *e.g.*, by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

[00132] Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, I. *et al.* (1997) *Nature* 385:810-813 and PCT International Publication Nos. WO 97/07668 and WO 97/07669. In brief, a cell, *e.g.*, a somatic cell, from the transgenic animal can be isolated and induced to exit the growth cycle and enter G_o phase. The quiescent cell can then be fused, *e.g.*, through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell, *e.g.*, the somatic cell, is isolated.

[00133] The 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or

32620 transgenic animals that express 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 mRNA or a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 peptide (detected immunocytochemically, using antibodies directed against 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 epitopes) at easily detectable levels should then be further evaluated to identify those animals which display characteristic pain.

Cell-Based Systems

[00134] Cells that contain and express 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene sequences which encode a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein, and, further, exhibit cellular phenotypes associated with nociception, may be used to identify compounds that exhibit analgesic effect. Such cells may include non-recombinant monocyte cell lines, such as U937 (ATCC# CRL-1593), THP-1 (ATCC#TIB-202), and P388D1 (ATCC# TIB-63); endothelial cells such as human umbilical vein endothelial cells (HUVECs), human microvascular endothelial cells (HMVEC), and bovine aortic endothelial cells (BAECs); as well as generic mammalian cell lines such as HeLa cells and COS cells, *e.g.*, COS-7 (ATCC# CRL-1651), and neural cell lines.. Further, such cells may include recombinant, transgenic cell lines. For example, the pain animal models of the invention, discussed above, may be used to generate cell lines, containing one or more cell types involved in nociception, that can be used as cell culture models for this disorder. While primary cultures derived from the pain model transgenic animals of the invention may be utilized, the generation of continuous cell lines is preferred. For examples of techniques which may be used to derive a continuous cell line from the transgenic animals, see Small *et al.*, (1985) *Mol. Cell Biol.* 5:642-648.

[00135] Alternatively, cells of a cell type known to be involved in nociception may be transfected with sequences capable of increasing or decreasing the amount of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921,

16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene expression within the cell. For example, 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene sequences may be introduced into, and overexpressed in, the genome of the cell of interest, or, if endogenous 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene sequences are present, they may be either overexpressed or, alternatively disrupted in order to underexpress or inactivate 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene expression.

[00136] In order to overexpress a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene, the coding portion of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene may be ligated to a regulatory sequence which is capable of driving gene expression in the cell type of interest, *e.g.*, an endothelial cell. Such regulatory regions will be well known to those of skill in the art, and may be utilized in the absence of undue experimentation. Recombinant methods for expressing target genes are described above.

[00137] For underexpression of an endogenous 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene sequence, such a sequence may be isolated and engineered such that when reintroduced into the genome of the cell type of interest, the endogenous 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 alleles will be inactivated. Preferably, the engineered 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 sequence is introduced via gene targeting such that the endogenous 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 sequence is disrupted upon integration of the engineered 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145,

59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 sequence into the cell's genome. Transfection of host cells with 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 genes is discussed, above.

[00138] Cells treated with compounds or transfected with 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 genes can be examined for phenotypes associated with nociception.

[00139] Transfection of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 nucleic acid may be accomplished by using standard techniques (described in, for example, Ausubel (1989) *supra*). Transfected cells should be evaluated for the presence of the recombinant 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene sequences, for expression and accumulation of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 mRNA, and for the presence of recombinant 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein production. In instances wherein a decrease in 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene expression is desired, standard techniques may be used to demonstrate whether a decrease in endogenous 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene expression and/or in 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein production is achieved.

[00140] Also provided are cells or a purified preparation thereof, e.g., human cells, in which an endogenous 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 is under the control of a regulatory sequence that does not normally control the expression of the endogenous 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410,

38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene. The expression characteristics of an endogenous gene within a cell, e.g., a cell line or microorganism, can be modified by inserting a heterologous DNA regulatory element into the genome of the cell such that the inserted regulatory element is operably linked to the endogenous 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene. For example, an endogenous 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene, e.g., a gene which is "transcriptionally silent," e.g., not normally expressed, or expressed only at very low levels, may be activated by inserting a regulatory element which is capable of promoting the expression of a normally expressed gene product in that cell. Techniques such as targeted homologous recombinations, can be used to insert the heterologous DNA as described in, e.g., Chappel, US 5,272,071; WO 91/06667, published on May 16, 1991.

Predictive Medicine:

[00141] The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the present invention relates to diagnostic assays for determining 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein and/or nucleic acid expression as well as 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity, in the context of a biological sample (*e.g.*, blood, serum, cells, *e.g.*, endothelial cells, or tissue, *e.g.*, vascular tissue) to thereby determine whether an individual is afflicted with a predisposition or is experiencing pain. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a painful disorder. For example, mutations in a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene can be assayed for in a biological sample. Such assays can be used for prognostic or predictive

purpose to thereby phophylactically treat an individual prior to the onset of a painful disorder.

[00142] Another aspect of the invention pertains to monitoring the influence of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 modulators (*e.g.*, anti-16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 antibodies or 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 ribozymes) on the expression or activity of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 in clinical trials.

[00143] These and other agents are described in further detail in the following sections.

Diagnostic Assays

[00144] To determine whether a subject is afflicted with a disease, a biological sample may be obtained from a subject and the biological sample may be contacted with a compound or an agent capable of detecting a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein or nucleic acid (*e.g.*, mRNA or genomic DNA) that encodes a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein, in the biological sample. A preferred agent for detecting 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 mRNA or genomic DNA. The nucleic acid probe can be, for example, the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 nucleic acid set forth in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43 or 45, or a portion thereof, such as an

oligonucleotide of at least 15, 20, 25, 30, 25, 40, 45, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

[00145] A preferred agent for detecting 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein in a sample is an antibody capable of binding to 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or F(ab')2) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin.

[00146] The term "biological sample" is intended to include tissues, cells, and biological fluids isolated from a subject, as well as tissues, cells, and fluids present within a subject. That is, the detection method of the invention can be used to detect 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. *In vitro* techniques for detection of

16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein include introducing into a subject a labeled anti-16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

[00147] In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein, mRNA, or genomic DNA, such that the presence of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein, mRNA or genomic DNA in the control sample with the presence of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein, mRNA or genomic DNA in the test sample.

Prognostic Assays

[00148] The present invention further pertains to methods for identifying subjects having or at risk of developing a disease associated with aberrant 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 expression or activity.

[00149] As used herein, the term "aberrant" includes a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 expression or activity which deviates

from the wild type 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 expression or activity. Aberrant expression or activity includes increased or decreased expression or activity, as well as expression or activity which does not follow the wild type developmental pattern of expression or the subcellular pattern of expression. For example, aberrant 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 expression or activity is intended to include the cases in which a mutation in the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene causes the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene to be under-expressed or over-expressed and situations in which such mutations result in a non-functional 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein or a protein which does not function in a wild-type fashion, *e.g.*, a protein which does not interact with a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 substrate, or one which interacts with a non-16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 substrate.

[00150] The assays described herein, such as the preceding diagnostic assays or the following assays, can be used to identify a subject having or at risk of developing a disease. A biological sample may be obtained from a subject and tested for the presence or absence of a genetic alteration. For example, such genetic alterations can be detected by ascertaining the existence of at least one of 1) a deletion of one or more nucleotides from a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene, 2) an addition of one or more nucleotides to a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene, 3) a substitution of one or more nucleotides of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene, 4) a

chromosomal rearrangement of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene, 5) an alteration in the level of a messenger RNA transcript of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene, 6) aberrant modification of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene, such as of the methylation pattern of the genomic DNA, 7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene, 8) a non-wild type level of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 -protein, 9) allelic loss of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene, and 10) inappropriate post-translational modification of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 -protein.

[00151] As described herein, there are a large number of assays known in the art which can be used for detecting genetic alterations in a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene. For example, a genetic alteration in a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene may be detected using a probe/primer in a polymerase chain reaction (PCR) (see, *e.g.*, U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, *e.g.*, Landegran *et al.* (1988) *Science* 241:1077-1080; and Nakazawa *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:360-364), the latter of which can be particularly useful for detecting point mutations in a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene (see Abravaya *et al.* (1995) *Nucleic Acids Res.* 23:675-682). This method includes collecting a biological sample from a subject, isolating nucleic acid (*e.g.*, genomic DNA, mRNA or both) from the sample,

contacting the nucleic acid sample with one or more primers which specifically hybridize to a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene under conditions such that hybridization and amplification of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

[00152] Alternative amplification methods include: self sustained sequence replication (Guatelli, J.C. *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh, D.Y. *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi, P.M. *et al.* (1988) *Bio-Technology* 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

[00153] In an alternative embodiment, mutations in a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene from a biological sample can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

[00154] In other embodiments, genetic mutations in 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 can be identified by hybridizing biological sample derived and control nucleic acids, *e.g.*, DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotide probes (Cronin, M.T. *et al.* (1996)

Human Mutation 7:244-255; Kozal, M.J. *et al.* (1996) *Nature Medicine* 2:753-759). For example, genetic mutations in 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, M.T. *et al.* (1996) *supra.* Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential, overlapping probes. This step allows for the identification of point mutations. This step is followed by a second hybridization array that allows for the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

[00155] In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene in a biological sample and detect mutations by comparing the sequence of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 in the biological sample with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxam and Gilbert (1977) *Proc. Natl. Acad. Sci. USA* 74:560) or Sanger (1977) *Proc. Natl. Acad. Sci. USA* 74:5463). It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays (Naeve, C. W. (1995) *Biotechniques* 19:448-53), including sequencing by mass spectrometry (see, *e.g.*, PCT International Publication No. WO 94/16101; Cohen *et al.* (1996) *Adv. Chromatogr.* 36:127-162; and Griffin *et al.* (1993) *Appl. Biochem. Biotechnol.* 38:147-159).

[00156] Other methods for detecting mutations in the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers *et al.* (1985) *Science* 230:1242). In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes formed by hybridizing (labeled) RNA or DNA containing the wild-type 16386, 15402, 21165, 1423, 636, 12303,

21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent which cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S1 nuclease to enzymatically digest the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. See, for example, Cotton *et al.* (1988) *Proc. Natl Acad Sci USA* 85:4397 and Saleeba *et al.* (1992) *Methods Enzymol.* 217:286-295. In a preferred embodiment, the control DNA or RNA can be labeled for detection.

[00157] In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662). According to an exemplary embodiment, a probe based on a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 sequence, *e.g.*, a wild-type 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. See, for example, U.S. Patent No. 5,459,039.

[00158] In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 genes. For example, single strand conformation polymorphism (SSCP) may be used

to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc Natl. Acad. Sci USA*: 86:2766; see also Cotton (1993) *Mutat. Res.* 285:125-144 and Hayashi (1992) *Genet. Anal. Tech. Appl.* 9:73-79). Single-stranded DNA fragments of sample and control 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen *et al.* (1991) *Trends Genet* 7:5).

[00159] In yet another embodiment the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers *et al.* (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to ensure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys Chem* 265:12753).

[00160] Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions which permit hybridization only if a perfect match is found (Saiki *et al.* (1986) *Nature* 324:163); Saiki *et al.* (1989) *Proc. Natl Acad. Sci USA* 86:6230). Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

[00161] Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant invention.

Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.* (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini *et al.* (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc. Natl. Acad. Sci USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

[00162] Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 modulator (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, or small molecule) to effectively treat a disease.

Monitoring of Effects During Clinical Trials

[00163] The present invention further provides methods for determining the effectiveness of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 modulator (*e.g.*, a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 modulator identified herein) in treating a disease. For example, the effectiveness of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 modulator in increasing 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene expression, protein levels, or in upregulating 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity, can be monitored in clinical trials of subjects exhibiting decreased 16386, 15402, 21165, 1423, 636,

12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene expression, protein levels, or downregulated 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity. Alternatively, the effectiveness of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 modulator in decreasing 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene expression, protein levels, or in downregulating 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity, can be monitored in clinical trials of subjects exhibiting increased 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene expression, protein levels, or 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity. In such clinical trials, the expression or activity of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene, and preferably, other genes that have been implicated in nociception can be used as a "read out" or marker of the phenotype of a particular cell.

[00164] For example, and not by way of limitation, genes, including 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 , that are modulated in cells by treatment with an agent which modulates 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents which modulate 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity on subjects suffering from a painful disorder in, for example, a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921,

16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 and other genes implicated in the painful disorder. The levels of gene expression (*e.g.*, a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods described herein, or by measuring the levels of activity of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 or other genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent which modulates 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity. This response state may be determined before, and at various points during treatment of the individual with the agent which modulates 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity.

[00165] In a preferred embodiment, the present invention provides a method for monitoring the effectiveness of treatment of a subject with an agent which modulates 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, or small molecule identified by the screening assays described herein) including the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein, mRNA, or genomic DNA in the pre-administration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein, mRNA, or genomic DNA in the pre-administration sample with the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913,

14303, 16816, 17827 or 32620 protein, mRNA, or genomic DNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 to higher levels than detected, *i.e.*, to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 to lower levels than detected, *i.e.* to decrease the effectiveness of the agent. According to such an embodiment, 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 expression or activity may be used as an indicator of the effectiveness of an agent, even in the absence of an observable phenotypic response.

Methods of Treatment:

[00166] The present invention provides for both prophylactic and therapeutic methods of treating a subject, *e.g.*, a human, at risk of (or susceptible to) a disease. With regard to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics. "Pharmacogenomics," as used herein, refers to the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term refers to the study of how a patient's genes determine his or her response to a drug (*e.g.*, a patient's "drug response phenotype", or "drug response genotype").

[00167] Thus, another aspect of the invention provides methods for tailoring an subject's prophylactic or therapeutic treatment with either the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 molecules of the present invention or 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 modulators according to that individual's drug response genotype. Pharmacogenomics allows a clinician or physician to target prophylactic or therapeutic treatments to patients

who will most benefit from the treatment and to avoid treatment of patients who will experience toxic drug-related side effects.

Prophylactic Methods

[00168] In one aspect, the invention provides a method for preventing in a subject, a disease by administering to the subject an agent which modulates 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 expression or 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity. Subjects at risk for a pain or painful disorder can be identified by, for example, any or a combination of the diagnostic or prognostic assays described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of aberrant 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 expression or activity, such that a disease is prevented or, alternatively, delayed in its progression. Depending on the type of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 aberrancy, for example, a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 agonist or 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

Therapeutic Methods

[00169] Described herein are methods and compositions whereby pain may be ameliorated. Certain painful disorders are brought about, at least in part, by an excessive level of a gene product, or by the presence of a gene product exhibiting an abnormal or excessive activity. As such, the reduction in the level and/or activity of such gene products would bring about the amelioration of pain. Techniques for the reduction of gene expression levels or the activity of a protein are discussed below.

[00170] Alternatively, certain other painful disorders are brought about, at least in part, by the absence or reduction of the level of gene expression, or a reduction in the level of a protein's activity. As such, an increase in the level of gene expression and/or the activity of such proteins would bring about the amelioration of pain.

[00171] In some cases, the up-regulation of a gene in a disease state reflects a protective role for that gene product in responding to the disease condition. Enhancement of such a gene's expression, or the activity of the gene product, will reinforce the protective effect it exerts. Some pain states may result from an abnormally low level of activity of such a protective gene. In these cases also, an increase in the level of gene expression and/or the activity of such gene products would bring about the amelioration of pain. Techniques for increasing target gene expression levels or target gene product activity levels are discussed herein.

[00172] Accordingly, another aspect of the invention pertains to methods of modulating 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 expression or activity for therapeutic purposes. Accordingly, in an exemplary embodiment, the modulatory method of the invention involves contacting a cell with a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 or agent that modulates one or more of the activities of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein activity associated with the cell (*e.g.*, an endothelial cell or an ovarian cell). An agent that modulates 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring target molecule of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein (*e.g.*, a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 ligand or substrate), a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 antibody, a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063,

57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 agonist or antagonist, a peptidomimetic of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 agonist or antagonist, or other small molecule. In one embodiment, the agent stimulates one or more 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activities. Examples of such stimulatory agents include active 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein and a nucleic acid molecule encoding 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 that has been introduced into the cell. In another embodiment, the agent inhibits one or more 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activities. Examples of such inhibitory agents include antisense 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 nucleic acid molecules, anti-16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 antibodies, and 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 inhibitors. These modulatory methods can be performed *in vitro* (*e.g.*, by culturing the cell with the agent) or, alternatively, *in vivo* (*e.g.*, by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant or unwanted expression or activity of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (*e.g.*, an agent identified by a screening assay described herein), or combination of agents that modulates (*e.g.*, upregulates or downregulates) 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 expression or activity. In another embodiment, the method involves administering a 16386, 15402, 21165, 1423,

636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein or nucleic acid molecule as therapy to compensate for reduced, aberrant, or unwanted 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 expression or activity.

[00173] Stimulation of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity is desirable in situations in which 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 is abnormally downregulated and/or in which increased 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity is likely to have a beneficial effect. Likewise, inhibition of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity is desirable in situations in which 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 is abnormally upregulated and/or in which decreased 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity is likely to have a beneficial effect.

Methods for Inhibiting Target Gene Expression, Synthesis, or Activity

[00174] As discussed above, genes involved in pain or painful disorders may cause such disorders via an increased level of gene activity. In some cases, such up-regulation may have a causative or exacerbating effect on the disease state. A variety of techniques may be used to inhibit the expression, synthesis, or activity of such genes and/or proteins.

[00175] For example, compounds such as those identified through assays described above, which exhibit inhibitory activity, may be used in accordance with the invention to ameliorate pain. Such molecules may include, but are not limited to, small organic molecules, peptides, antibodies, and the like.

[00176] For example, compounds can be administered that compete with endogenous ligand for the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063,

57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein. The resulting reduction in the amount of ligand-bound 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein will modulate endothelial cell physiology. Compounds that can be particularly useful for this purpose include, for example, soluble proteins or peptides, such as peptides comprising one or more of the extracellular domains, or portions and/or analogs thereof, of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein, including, for example, soluble fusion proteins such as Ig-tailed fusion proteins. (For a discussion of the production of Ig-tailed fusion proteins, see, for example, U.S. Pat. No. 5,116,964). Alternatively, compounds, such as ligand analogs or antibodies, that bind to the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 receptor site, but do not activate the protein, (*e.g.*, receptor-ligand antagonists) can be effective in inhibiting 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein activity.

[00177] Further, antisense and ribozyme molecules which inhibit expression of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene may also be used in accordance with the invention to inhibit aberrant 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene activity. Still further, triple helix molecules may be utilized in inhibiting aberrant 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene activity.

[00178] The antisense nucleic acid molecules used in the methods of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein to thereby inhibit expression of the protein, *e.g.*, by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an

antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention include direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

[00179] In yet another embodiment, an antisense nucleic acid molecule used in the methods of the invention is an α-anomeric nucleic acid molecule. An α-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gaultier *et al.* (1987) *Nucleic Acids. Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.* (1987) *FEBS Lett.* 215:327-330).

[00180] In still another embodiment, an antisense nucleic acid used in the methods of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 mRNA transcripts to thereby inhibit translation of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 mRNA. A ribozyme having specificity for a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620-encoding nucleic acid can be designed based upon the nucleotide sequence of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914,

94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 cDNA disclosed herein (*i.e.*, SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43 or 45). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620-encoding mRNA (see, for example, Cech *et al.* U.S. Patent No. 4,987,071; and Cech *et al.* U.S. Patent No. 5,116,742). Alternatively, 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules (see, for example, Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418).

[00181] 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene expression can also be inhibited by targeting nucleotide sequences complementary to the regulatory region of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 (*e.g.*, the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 promoter and/or enhancers) to form triple helical structures that prevent transcription of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene in target cells (see, for example, Helene, C. (1991) *Anticancer Drug Des.* 6(6):569-84; Helene, C. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L.J. (1992) *Bioassays* 14(12):807-15).

[00182] Antibodies that are both specific for the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein and interfere with its activity may also be used to modulate or inhibit 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein function. Such antibodies may be generated using standard techniques described herein, against the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913,

14303, 16816, 17827 or 32620 protein itself or against peptides corresponding to portions of the protein. Such antibodies include but are not limited to polyclonal, monoclonal, Fab fragments, single chain antibodies, or chimeric antibodies.

[00183] In instances where the target gene protein is intracellular and whole antibodies are used, internalizing antibodies may be preferred. Lipofectin liposomes may be used to deliver the antibody or a fragment of the Fab region which binds to the target epitope into cells. Where fragments of the antibody are used, the smallest inhibitory fragment which binds to the target protein's binding domain is preferred. For example, peptides having an amino acid sequence corresponding to the domain of the variable region of the antibody that binds to the target gene protein may be used. Such peptides may be synthesized chemically or produced via recombinant DNA technology using methods well known in the art (described in, for example, Creighton (1983), *supra*; and Sambrook *et al.* (1989) *supra*). Single chain neutralizing antibodies which bind to intracellular target gene epitopes may also be administered. Such single chain antibodies may be administered, for example, by expressing nucleotide sequences encoding single-chain antibodies within the target cell population by utilizing, for example, techniques such as those described in Marasco *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:7889-7893).

[00184] In some instances, the target gene protein is extracellular, or is a transmembrane protein, such as the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein. Antibodies that are specific for one or more extracellular domains of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein, for example, and that interfere with its activity, are particularly useful in treating pain or a painful disorder. Such antibodies are especially efficient because they can access the target domains directly from the bloodstream. Any of the administration techniques described below which are appropriate for peptide administration may be utilized to effectively administer inhibitory target gene antibodies to their site of action.

Methods for Restoring or Enhancing Target Gene Activity

[00185] Genes that cause pain may be underexpressed within pain or painful disorders. Alternatively, the activity of the protein products of such genes may be decreased, leading to the development of pain. Such down-regulation of gene expression or

decrease of protein activity might have a causative or exacerbating effect on the disease state.

[00186] In some cases, genes that are up-regulated in the disease state might be exerting a protective effect. A variety of techniques may be used to increase the expression, synthesis, or activity of genes and/or proteins that exert a protective effect in response to pain conditions.

[00187] Described in this section are methods whereby the level 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity may be increased to levels wherein pain are ameliorated. The level of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity may be increased, for example, by either increasing the level of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene expression or by increasing the level of active 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein which is present.

[00188] For example, a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein, at a level sufficient to ameliorate pain may be administered to a patient exhibiting such symptoms. Any of the techniques discussed below may be used for such administration. One of skill in the art will readily know how to determine the concentration of effective, non-toxic doses of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein, utilizing techniques such as those described below.

[00189] Additionally, RNA sequences encoding a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein may be directly administered to a patient exhibiting pain, at a concentration sufficient to produce a level of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein such that pain

are ameliorated. Any of the techniques discussed below, which achieve intracellular administration of compounds, such as, for example, liposome administration, may be used for the administration of such RNA molecules. The RNA molecules may be produced, for example, by recombinant techniques such as those described herein.

[00190] Further, subjects may be treated by gene replacement therapy. One or more copies of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene, or a portion thereof, that directs the production of a normal 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein with 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 function, may be inserted into cells using vectors which include, but are not limited to adenovirus, adeno-associated virus, and retrovirus vectors, in addition to other particles that introduce DNA into cells, such as liposomes. Additionally, techniques such as those described above may be used for the introduction of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene sequences into human cells.

[00191] Cells, preferably, autologous cells, containing 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 expressing gene sequences may then be introduced or reintroduced into the subject at positions which allow for the amelioration of pain. Such cell replacement techniques may be preferred, for example, when the gene product is a secreted, extracellular gene product.

Pharmaceutical Compositions

[00192] Another aspect of the invention pertains to methods for treating a subject suffering from a disease. These methods involve administering to a subject an agent which modulates 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 expression or activity (*e.g.*, an agent identified by a screening assay described herein), or a combination of such agents. In another embodiment, the method involves administering to a subject a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063,

57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein or nucleic acid molecule as therapy to compensate for reduced, aberrant, or unwanted 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 expression or activity.

[00193] Stimulation of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity is desirable in situations in which 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 is abnormally downregulated and/or in which increased 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity is likely to have a beneficial effect. Likewise, inhibition of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity is desirable in situations in which 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 is abnormally upregulated and/or in which decreased 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity is likely to have a beneficial effect.

[00194] The agents which modulate 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity can be administered to a subject using pharmaceutical compositions suitable for such administration. Such compositions typically comprise the agent (*e.g.*, nucleic acid molecule, protein, or antibody) and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is

contemplated. Supplementary active compounds can also be incorporated into the compositions.

[00195] A pharmaceutical composition used in the therapeutic methods of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[00196] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, and sodium chloride in the composition. Prolonged absorption of the injectable

compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

[00197] Sterile injectable solutions can be prepared by incorporating the agent that modulates 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity (*e.g.*, a fragment of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein or an anti-16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[00198] Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[00199] For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

[00200] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

[00201] The agents that modulate 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

[00202] In one embodiment, the agents that modulate 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

[00203] It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the agent that modulates 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921,

16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an agent for the treatment of subjects.

[00204] Toxicity and therapeutic efficacy of such agents can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and can be expressed as the ratio LD50/ED50. Agents which exhibit large therapeutic indices are preferred. While agents that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such agents to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

[00205] The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 modulating agents lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any agent used in the therapeutic methods of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

[00206] As defined herein, a therapeutically effective amount of protein or polypeptide (*i.e.*, an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of

the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a protein, polypeptide, or antibody can include a single treatment or, preferably, can include a series of treatments.

[00207] In a preferred example, a subject is treated with antibody, protein, or polypeptide in the range of between about 0.1 to 20 mg/kg body weight, one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. It will also be appreciated that the effective dosage of antibody, protein, or polypeptide used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage may result and become apparent from the results of diagnostic assays as described herein.

[00208] The present invention encompasses agents which modulate expression or activity. An agent may, for example, be a small molecule. For example, such small molecules include, but are not limited to, peptides, peptidomimetics, amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (*i.e*,. including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds. It is understood that appropriate doses of small molecule agents depends upon a number of factors within the ken of the ordinarily skilled physician, veterinarian, or researcher. The dose(s) of the small molecule will vary, for example, depending upon the identity, size, and condition of the subject or sample being treated, further depending upon the route by which the composition is to be administered, if applicable, and the effect which the practitioner desires the small molecule to have upon the nucleic acid or polypeptide of the invention.

[00209] Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (*e.g.*, about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram). It is furthermore understood that appropriate doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated. Such appropriate doses may be determined using the assays described herein.

When one or more of these small molecules is to be administered to an animal (*e.g.*, a human) in order to modulate expression or activity of a polypeptide or nucleic acid of the invention, a physician, veterinarian, or researcher may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

[00210] Further, an antibody (or fragment thereof) may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive metal ion. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and vinblastine).

[00211] The conjugates of the invention can be used for modifying a given biological response, the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, alpha-interferon, beta-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; or biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte

macrophase colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

[00212] Techniques for conjugating such therapeutic moiety to antibodies are well known, see, *e.g.*, Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin *et al.* (eds.), pp. 303-16 (Academic Press 1985), and Thorpe *et al.*, "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62:119-58 (1982). Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980.

[00213] The nucleic acid molecules used in the methods of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see, *e.g.*, Chen *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

Pharmacogenomics

[00214] In conjunction with the therapeutic methods of the invention, pharmacogenomics (*i.e.*, the study of the relationship between a subject's genotype and that subject's response to a foreign compound or drug) may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus,

a physician or clinician may consider applying knowledge obtained in relevant pharmacogenomics studies in determining whether to administer an agent which modulates 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity, as well as tailoring the dosage and/or therapeutic regimen of treatment with an agent which modulates 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity.

[00215] Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See, for example, Eichelbaum, M. *et al.* (1996) *Clin. Exp.Pharmacol. Physiol.* 23(10-11): 983-985 and Linder, M.W. *et al.* (1997) *Clin. Chem.* 43(2):254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare genetic defects or as naturally-occurring polymorphisms. For example, glucose-6-phosphate aminopeptidase deficiency (G6PD) is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

[00216] One pharmacogenomics approach to identifying genes that predict drug response, known as "a genome-wide association", relies primarily on a high-resolution map of the human genome consisting of already known gene-related markers (*e.g.*, a "bi-allelic" gene marker map which consists of 60,000-100,000 polymorphic or variable sites on the human genome, each of which has two variants). Such a high-resolution genetic map can be compared to a map of the genome of each of a statistically significant number of patients taking part in a Phase II/III drug trial to identify markers associated with a particular observed drug response or side effect. Alternatively, such a high resolution map can be generated from a combination of some ten million known single nucleotide polymorphisms (SNPs) in the human genome. As used herein, a "SNP" is a common alteration that occurs in a single nucleotide base in a stretch of DNA. For example, a SNP may occur once per every 1000 bases of DNA. A SNP may be involved in a disease process, however, the vast majority may not be disease-associated. Given a genetic map based on the occurrence of

such SNPs, individuals can be grouped into genetic categories depending on a particular pattern of SNPs in their individual genome. In such a manner, treatment regimens can be tailored to groups of genetically similar individuals, taking into account traits that may be common among such genetically similar individuals.

[00217] Alternatively, a method termed the "candidate gene approach" can be utilized to identify genes that predict drug response. According to this method, if a gene that encodes a drug target is known (*e.g.*, a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein used in the methods of the present invention), all common variants of that gene can be fairly easily identified in the population and it can be determined if having one version of the gene versus another is associated with a particular drug response.

[00218] As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (*e.g.*, N-acetyltransferase 2 (NAT 2) and the cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

[00219] Alternatively, a method termed the "gene expression profiling" can be utilized to identify genes that predict drug response. For example, the gene expression of an animal dosed with a drug (*e.g.*, a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303,

16816, 17827 or 32620 molecule or 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 modulator used in the methods of the present invention) can give an indication whether gene pathways related to toxicity have been turned on.

[00220] Information generated from more than one of the above pharmacogenomics approaches can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment of a subject. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and, thus, enhance therapeutic or prophylactic efficiency when treating a subject suffering from pain or painful disorders with an agent which modulates 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity.

Recombinant Expression Vectors and Host Cells Used in the Methods of the Invention

[00221] The methods of the invention (*e.g.*, the screening assays described herein) include the use of vectors, preferably expression vectors, containing a nucleic acid encoding a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein (or a portion thereof). As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*,

replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

[00222] The recombinant expression vectors to be used in the methods of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel (1990) *Methods Enzymol.* 185:3-7. Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cells and those which direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the like. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (*e.g.*, 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 proteins, mutant forms of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 proteins, fusion proteins, and the like).

[00223] The recombinant expression vectors to be used in the methods of the invention can be designed for expression of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 proteins in prokaryotic or eukaryotic cells. For example, 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 proteins can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression

vectors), yeast cells, or mammalian cells. Suitable host cells are discussed further in Goeddel (1990) *supra*. Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

[00224] Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

[00225] Purified fusion proteins can be utilized in 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity assays, (*e.g.*, direct assays or competitive assays described in detail below), or to generate antibodies specific for 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 proteins. In a preferred embodiment, a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 fusion protein expressed in a retroviral expression vector of the present invention can be utilized to infect bone marrow cells which are subsequently transplanted into irradiated recipients. The pathology of the subject recipient is then examined after sufficient time has passed (*e.g.*, six weeks).

[00226] In another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian

expression vectors include pCDM8 (Seed, B. (1987) *Nature* 329:840) and pMT2PC (Kaufman *et al.* (1987) *EMBO J.* 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook, J. *et al.*, *Molecular Cloning: A Laboratory Manual. 2nd ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

[00227] In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid).

[00228] The methods of the invention may further use a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific, or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid, or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes, see Weintraub, H. *et al.*, Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) 1986.

[00229] Another aspect of the invention pertains to the use of host cells into which a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 nucleic acid molecule of the invention is introduced, *e.g.*, a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911,

85913, 14303, 16816, 17827 or 32620 nucleic acid molecule within a recombinant expression vector or a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 nucleic acid molecule containing sequences which allow it to homologously recombine into a specific site of the host cell's genome. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

[00230] A host cell can be any prokaryotic or eukaryotic cell. For example, a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

[00231] Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook *et al.* (*Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989), and other laboratory manuals.

[00232] A host cell used in the methods of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein. Accordingly, the invention further provides methods for producing a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of the invention (into which a

recombinant expression vector encoding a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein has been introduced) in a suitable medium such that a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein is produced. In another embodiment, the method further comprises isolating a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein from the medium or the host cell.

Isolated Nucleic Acid Molecules Used in the Methods of the Invention

[00233] The methods of the invention include the use of isolated nucleic acid molecules that encode 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 proteins or biologically active portions thereof, as well as nucleic acid fragments sufficient for use as hybridization probes to identify 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 -encoding nucleic acid molecules (e.g., 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 mRNA) and fragments for use as PCR primers for the amplification or mutation of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA) and RNA molecules (*e.g.*, mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

[00234] A nucleic acid molecule used in the methods of the present invention, *e.g.*, a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43 or 45, or a portion thereof, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or portion of the nucleic acid sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43 or 45, as a hybridization probe,

16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 nucleic acid molecules can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

[00235] Moreover, a nucleic acid molecule encompassing all or a portion of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43 or 45 can be isolated by the polymerase chain reaction (PCR) using synthetic oligonucleotide primers designed based upon the sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43 or 45.

[00236] A nucleic acid used in the methods of the invention can be amplified using cDNA, mRNA or, alternatively, genomic DNA as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. Furthermore, oligonucleotides corresponding to 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

[00237] In a preferred embodiment, the isolated nucleic acid molecules used in the methods of the invention comprise the nucleotide sequence shown in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43 or 45, a complement of the nucleotide sequence shown in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43 or 45, or a portion of any of these nucleotide sequences. A nucleic acid molecule which is complementary to the nucleotide sequence shown in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43 or 45, is one which is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43 or 45, such that it can hybridize to the nucleotide sequence shown in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43 or 45, thereby forming a stable duplex.

[00238] In still another preferred embodiment, an isolated nucleic acid molecule used in the methods of the present invention comprises a nucleotide sequence which is at least about 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more identical to the entire length of the nucleotide sequence shown in SEQ ID NO:1, 3, 5, 7, 9,

11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43 or 45, or a portion of any of this nucleotide sequence.

[00239] Moreover, the nucleic acid molecules used in the methods of the invention can comprise only a portion of the nucleic acid sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43 or 45, for example, a fragment which can be used as a probe or primer or a fragment encoding a portion of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein, *e.g.*, a biologically active portion of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein. The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12 or 15, preferably about 20 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, or 75 consecutive nucleotides of a sense sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43 or 45, of an anti-sense sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43 or 45, or of a naturally occurring allelic variant or mutant of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43 or 45. In one embodiment, a nucleic acid molecule used in the methods of the present invention comprises a nucleotide sequence which is greater than 100, 100-200, 200-300, 300-400, 400-500, 500-600, 600-700, 700-800, 800-900, 900-1000, 1000-1100, 1100-1200, 1200-1300, or more nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic acid molecule of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43 or 45.

[00240] As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences that are significantly identical or homologous to each other remain hybridized to each other. Preferably, the conditions are such that sequences at least about 70%, more preferably at least about 80%, even more preferably at least about 85% or 90% identical to each other remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, Ausubel *et al.*, eds., John Wiley & Sons, Inc. (1995), sections 2, 4 and 6. Additional stringent conditions can be found in *Molecular Cloning: A Laboratory Manual*, Sambrook *et al.*, Cold Spring Harbor

Press, Cold Spring Harbor, NY (1989), chapters 7, 9 and 11. A preferred, non-limiting example of stringent hybridization conditions includes hybridization in 4X sodium chloride/sodium citrate (SSC), at about 65-70°C (or hybridization in 4X SSC plus 50% formamide at about 42-50°C) followed by one or more washes in 1X SSC, at about 65-70° C. A preferred, non-limiting example of highly stringent hybridization conditions includes hybridization in 1X SSC, at about 65-70°C (or hybridization in 1X SSC plus 50% formamide at about 42-50°C) followed by one or more washes in 0.3X SSC, at about 65-70° C. A preferred, non-limiting example of reduced stringency hybridization conditions includes hybridization in 4X SSC, at about 50-60°C (or alternatively hybridization in 6X SSC plus 50% formamide at about 40-45°C) followed by one or more washes in 2X SSC, at about 50-60°C. Ranges intermediate to the above-recited values, *e.g.*, at 65-70°C or at 42-50°C are also intended to be encompassed by the present invention. SSPE (1xSSPE is 0.15M NaCl, 10mM NaH_2PO_4, and 1.25mM EDTA, pH 7.4) can be substituted for SSC (1xSSC is 0.15M NaCl and 15mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 minutes each after hybridization is complete. The hybridization temperature for hybrids anticipated to be less than 50 base pairs in length should be 5-10°C less than the melting temperature (T_m) of the hybrid, where T_m is determined according to the following equations. For hybrids less than 18 base pairs in length, $T_m(°C) = 2(\#\text{ of A + T bases}) + 4(\#\text{ of G + C bases})$. For hybrids between 18 and 49 base pairs in length, $T_m(°C) = 81.5 + 16.6(\log_{10}[Na^+]) + 0.41(\%G+C) - (600/N)$, where N is the number of bases in the hybrid, and $[Na^+]$ is the concentration of sodium ions in the hybridization buffer ($[Na^+]$ for 1xSSC = 0.165 M). It will also be recognized by the skilled practitioner that additional reagents may be added to hybridization and/or wash buffers to decrease non-specific hybridization of nucleic acid molecules to membranes, for example, nitrocellulose or nylon membranes, including but not limited to blocking agents (*e.g.*, BSA or salmon or herring sperm carrier DNA), detergents (*e.g.*, SDS), chelating agents (*e.g.*, EDTA), Ficoll, PVP and the like. When using nylon membranes, in particular, an additional preferred, non-limiting example of stringent hybridization conditions is hybridization in 0.25-0.5M NaH_2PO_4, 7% SDS at about 65°C, followed by one or more washes at 0.02M NaH_2PO_4, 1% SDS at 65°C, see *e.g.*, Church and Gilbert (1984) *Proc. Natl. Acad. Sci. USA* 81:1991-1995, (or alternatively 0.2X SSC, 1% SDS).

[00241] In preferred embodiments, the probe further comprises a label group attached thereto, *e.g.*, the label group can be a radioisotope, a fluorescent compound, an enzyme, or

an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissue which misexpress a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein, such as by measuring a level of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 -encoding nucleic acid in a sample of cells from a subject *e.g.*, detecting 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 mRNA levels or determining whether a genomic 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene has been mutated or deleted.

[00242] The methods of the invention further encompass the use of nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43 or 45, due to degeneracy of the genetic code and thus encode the same 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 proteins as those encoded by the nucleotide sequence shown in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43 or 45. In another embodiment, an isolated nucleic acid molecule included in the methods of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44 or 46.

[00243] The methods of the invention further include the use of allelic variants of human 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620, *e.g.*, functional and non-functional allelic variants. Functional allelic variants are naturally occurring amino acid sequence variants of the human 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein that maintain a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity. Functional allelic variants will typically contain only conservative substitution of one or more amino acids of

SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44 or 46, or substitution, deletion or insertion of non-critical residues in non-critical regions of the protein.

[00244] Non-functional allelic variants are naturally occurring amino acid sequence variants of the human 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein that do not have a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity. Non-functional allelic variants will typically contain a non-conservative substitution, deletion, or insertion or premature truncation of the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44 or 46, or a substitution, insertion or deletion in critical residues or critical regions of the protein.

[00245] The methods of the present invention may further use non-human orthologues of the human 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein. Orthologues of the human 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein are proteins that are isolated from non-human organisms and possess the same 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity.

[00246] The methods of the present invention further include the use of nucleic acid molecules comprising the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43 or 45, or a portion thereof, in which a mutation has been introduced. The mutation may lead to amino acid substitutions at "non-essential" amino acid residues or at "essential" amino acid residues. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 (*e.g.*, the sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44 or 46) without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are

conserved among the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 proteins of the present invention are not likely to be amenable to alteration.

[00247] Mutations can be introduced into SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43 or 45, by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43 or 45, the encoded protein can be expressed recombinantly and the activity of the protein can be determined using the assay described herein.

[00248] Another aspect of the invention pertains to the use of isolated nucleic acid molecules which are antisense to the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43 or 45. An "antisense" nucleic acid comprises a nucleotide sequence which is complementary to a "sense" nucleic acid

encoding a protein, *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 coding strand, or to only a portion thereof. In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues. In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (also referred to as 5' and 3' untranslated regions).

[00249] Given the coding strand sequences encoding 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 disclosed herein, antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical

synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest). Antisense nucleic acid molecules used in the methods of the invention are further described above, in section IV.

[00250] In yet another embodiment, the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 nucleic acid molecules used in the methods of the present invention can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to generate peptide nucleic acids (see Hyrup B. *et al.* (1996) *Bioorganic & Medicinal Chemistry* 4 (1): 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, *e.g.*, DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral

backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *et al.* (1996) *Proc. Natl. Acad. Sci.* 93:14670-675.

[00251] PNAs of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 nucleic acid molecules can be used in the therapeutic and diagnostic applications described herein. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNAs of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (*e.g.*, by PNA-directed PCR clamping); as 'artificial restriction enzymes' when used in combination with other enzymes, (*e.g.*, S1 nucleases (Hyrup B. *et al.* (1996) *supra*)); or as probes or primers for DNA sequencing or hybridization (Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *et al.* (1996) *supra*).

[00252] In another embodiment, PNAs of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 can be modified, (*e.g.*, to enhance their stability or cellular uptake), by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 nucleic acid molecules can be generated which may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, (*e.g.*, RNAse H and DNA polymerases), to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup B. *et al.* (1996) *supra*). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup B. *et al.* (1996) *supra* and Finn P.J. *et al.* (1996) *Nucleic Acids Res.* 24 (17): 3357-63. For example, a DNA chain can be synthesized on a solid support using standard

phosphoramidite coupling chemistry and modified nucleoside analogs, *e.g.*, 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used as a between the PNA and the 5' end of DNA (Mag, M. *et al.* (1989) *Nucleic Acid Res.* 17: 5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn P.J. *et al.* (1996) *supra*). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment (Peterser, K.H. *et al.* (1975) *Bioorganic Med. Chem. Lett.* 5: 1119-11124).

[00253] In other embodiments, the oligonucleotide used in the methods of the invention may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:6553-6556; Lemaitre *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652; PCT Publication No. W088/09810) or the blood-brain barrier (see, *e.g.*, PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (See, *e.g.*, Krol *et al.* (1988) *Bio-Techniques* 6:958-976) or intercalating agents. (See, *e.g.*, Zon (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, (*e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

Isolated 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 Proteins and Anti-16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 Antibodies Used in the Methods of the Invention

[00254] The methods of the invention include the use of isolated 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 proteins, and biologically active portions thereof, as well as polypeptide fragments suitable for use as immunogens to raise anti-16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 antibodies. In one embodiment, native 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 proteins can be isolated from cells or tissue

sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

[00255] As used herein, a "biologically active portion" of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein includes a fragment of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein having a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity. Biologically active portions of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein include peptides comprising amino acid sequences sufficiently identical to or derived from the amino acid sequence of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein, *e.g.*, the amino acid sequence shown in SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44 or 46, which include fewer amino acids than the full length 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 proteins, and exhibit at least one activity of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein (*e.g.*, the N-terminal region of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein that is believed

to be involved in the regulation of apoptotic activity). A biologically active portion of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein can be a polypeptide which is, for example, 25, 50, 75, 100, 125, 150, 175, 200, 250, 300 or more amino acids in length. Biologically active portions of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein can be used as targets for developing agents which modulate a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 activity.

[00256] In a preferred embodiment, the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein used in the methods of the invention has an amino acid sequence shown in SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44 or 46. In other embodiments, the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein is substantially identical to SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44 or 46, and retains the functional activity of the protein of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44 or 46, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail in subsection V above. Accordingly, in another embodiment, the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein used in the methods of the invention is a protein which comprises an amino acid sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44 or 46.

[00257] To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at

least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, or 90% of the length of the reference sequence (*e.g.*, when aligning a second sequence to the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44 or 46 having 500 amino acid residues, at least 75, preferably at least 150, more preferably at least 225, even more preferably at least 300, and even more preferably at least 400 or more amino acid residues are aligned). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

[00258] The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* 48:444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package using either a Blosum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the algorithm of E. Meyers and W. Miller (*Comput. Appl. Biosci.* 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0 or 2.0U), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

[00259] The methods of the invention may also use 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 chimeric or fusion proteins. As used herein, a

16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 "chimeric protein" or "fusion protein" comprises a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 polypeptide operatively linked to a non-16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 polypeptide. An "16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 molecule, whereas a "non-16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein which is not substantially homologous to the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein, *e.g.*, a protein which is different from the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein and which is derived from the same or a different organism. Within a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 fusion protein the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 polypeptide can correspond to all or a portion of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein. In a preferred embodiment, a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 fusion protein comprises at least one biologically active portion of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein. In another preferred embodiment, a

16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 fusion protein comprises at least two biologically active portions of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein. Within the fusion protein, the term "operatively linked" is intended to indicate that the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 polypeptide and the non-16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 polypeptide are fused in-frame to each other. The non-16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 polypeptide can be fused to the N-terminus or C-terminus of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 polypeptide.

[00260] For example, in one embodiment, the fusion protein is a GST-16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 fusion protein in which the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 .

[00261] In another embodiment, this fusion protein is a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein containing a heterologous signal sequence at its N-terminus. In certain host cells (*e.g.*, mammalian host cells), expression and/or secretion of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 can be increased through use of a heterologous signal sequence.

[00262] The 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 fusion proteins used in the methods of the invention can be incorporated into pharmaceutical compositions and administered to a subject *in vivo*. The 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 fusion proteins can be used to affect the bioavailability of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 substrate. Use of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 fusion proteins may be useful therapeutically for the treatment of disorders caused by, for example, (i) aberrant modification or mutation of a gene encoding a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein; (ii) mis-regulation of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 gene; and (iii) aberrant post-translational modification of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein.

[00263] Moreover, the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 -fusion proteins used in the methods of the invention can be used as immunogens to produce anti-16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 antibodies in a subject, to purify 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 ligands and in screening assays to identify molecules which inhibit the interaction of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 with a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 substrate.

[00264] Preferably, a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 chimeric or fusion protein used in the methods of the invention is produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, *Current Protocols in Molecular Biology*, eds. Ausubel *et al.* John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide). A 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 -encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein.

[00265] The present invention also pertains to the use of variants of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 proteins which function as either 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 agonists (mimetics) or as 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 antagonists. Variants of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 proteins can be generated by mutagenesis, *e.g.*, discrete point mutation or truncation of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913,

14303, 16816, 17827 or 32620 protein. An agonist of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 proteins can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein. An antagonist of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein can inhibit one or more of the activities of the naturally occurring form of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein by, for example, competitively modulating a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 -mediated activity of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein.

[00266] In one embodiment, variants of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein which function as either 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 agonists (mimetics) or as 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 antagonists can be identified by screening combinatorial libraries of mutants, *e.g.*, truncation mutants, of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein for 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063,

57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein agonist or antagonist activity. In one embodiment, a variegated library of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display) containing the set of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 sequences therein. There are a variety of methods which can be used to produce libraries of potential 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, *e.g.*, Narang, S.A. (1983) *Tetrahedron* 39:3; Itakura *et al.* (1984) *Annu. Rev. Biochem.* 53:323; Itakura *et al.* (1984) *Science* 198:1056; Ike *et al.* (1983) *Nucleic Acid Res.* 11:477).

[00267] In addition, libraries of fragments of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein coding sequence can be used to generate a variegated population of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 fragments for screening and subsequent selection of variants of a

16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal, C-terminal and internal fragments of various sizes of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein.

[00268] Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 proteins. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 variants (Arkin and Yourvan (1992) *Proc. Natl. Acad. Sci. USA* 89:7811-7815; Delgrave *et al.* (1993) *Protein Engineering* 6(3):327-331).

[00269] The methods of the present invention further include the use of anti-16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914,

94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 antibodies. An isolated 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 using standard techniques for polyclonal and monoclonal antibody preparation. A full-length 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein can be used or, alternatively, antigenic peptide fragments of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 can be used as immunogens. The antigenic peptide of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 comprises at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44 or 46 and encompasses an epitope of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 such that an antibody raised against the peptide forms a specific immune complex with the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein. Preferably, the antigenic peptide comprises at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues.

[00270] Preferred epitopes encompassed by the antigenic peptide are regions of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 that are located on the surface of the protein, *e.g.*, hydrophilic regions, as well as regions with high antigenicity.

[00271] A 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 immunogen is typically used to prepare antibodies by immunizing a suitable subject, (*e.g.*, rabbit, goat, mouse, or other mammal) with the immunogen. An appropriate

immunogenic preparation can contain, for example, recombinantly expressed 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein or a chemically synthesized 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 polypeptide. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 preparation induces a polyclonal anti-16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 antibody response.

[00272] The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site which specifically binds (immunoreacts with) an antigen, such as a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and $F(ab')_2$ fragments which can be generated by treating the antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies that bind 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 molecules. The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 . A monoclonal antibody composition thus typically displays a single binding affinity for a particular 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein with which it immunoreacts.

[00273] Polyclonal anti-16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 antibodies can be prepared as described above by immunizing a

suitable subject with a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 immunogen. The anti-16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 . If desired, the antibody molecules directed against 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 can be isolated from the mammal (*e.g.*, from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization, *e.g.*, when the anti-16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256:495-497) (see also, Brown *et al.* (1981) *J. Immunol.* 127:539-46; Brown *et al.* (1980) *J. Biol. Chem.* 255:4980-83; Yeh *et al.* (1976) *Proc. Natl. Acad. Sci. USA* 76:2927-31; and Yeh *et al.* (1982) *Int. J. Cancer* 29:269-75), the more recent human B cell hybridoma technique (Kozbor *et al.* (1983) *Immunol Today* 4:72), the EBV-hybridoma technique (Cole *et al.* (1985) *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing monoclonal antibody hybridomas is well known (see generally Kenneth, R. H. in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, New York (1980); Lerner, E. A. (1981) *Yale J. Biol. Med.* 54:387-402; Gefter, M. L. *et al.* (1977) *Somatic Cell Genet.* 3:231-36). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with a 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds 16386, 15402, 21165, 1423, 636, 12303, 21425,

27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 .

[00274] Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating an anti-16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 monoclonal antibody (see, *e.g.*, G. Galfre *et al.* (1977) *Nature* 266:55052; Gefter *et al.* (1977) *supra*; Lerner (1981) *supra*; and Kenneth (1980) *supra*). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods which also would be useful. Typically, the immortal cell line (*e.g.*, a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine hybridomas can be made by fusing lymphocytes from a mouse immunized with an immunogenic preparation of the present invention with an immortalized mouse cell line. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). Any of a number of myeloma cell lines can be used as a fusion partner according to standard techniques, *e.g.*, the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from ATCC. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 , *e.g.*, using a standard ELISA assay.

[00275] Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal anti-16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (*e.g.*, an antibody phage display library) with 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 to thereby isolate immunoglobulin library members that bind 16386, 15402, 21165, 1423, 636, 12303, 21425,

27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 . Kits for generating and screening phage display libraries are commercially available (*e.g.*, the Pharmacia *Recombinant Phage Antibody System*, Catalog No. 27-9400-01; and the Stratagene *SurfZAP™ Phage Display Kit*, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, Ladner *et al.* U.S. Patent No. 5,223,409; Kang *et al.* PCT International Publication No. WO 92/18619; Dower *et al.* PCT International Publication No. WO 91/17271; Winter *et al.* PCT International Publication WO 92/20791; Markland *et al.* PCT International Publication No. WO 92/15679; Breitling *et al.* PCT International Publication WO 93/01288; McCafferty *et al.* PCT International Publication No. WO 92/01047; Garrard *et al.* PCT International Publication No. WO 92/09690; Ladner *et al.* PCT International Publication No. WO 90/02809; Fuchs *et al.* (1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992) *Hum. Antibod. Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; Griffiths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.* (1992) *J. Mol. Biol.* 226:889-896; Clarkson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:3576-3580; Garrad *et al.* (1991) *Bio/Technology* 9:1373-1377; Hoogenboom *et al.* (1991) *Nuc. Acid Res.* 19:4133-4137; Barbas *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:7978-7982; and McCafferty *et al.* (1990) *Nature* 348:552-554.

[00276] Additionally, recombinant anti-16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the methods of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in Robinson *et al.* International Application No. PCT/US86/02269; Akira, *et al.* European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison *et al.* European Patent Application 173,494; Neuberger *et al.* PCT International Publication No. WO 86/01533; Cabilly *et al.* U.S. Patent No. 4,816,567; Cabilly *et al.* European Patent Application 125,023; Better *et al.* (1988) *Science* 240:1041-1043; Liu *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:3439-3443; Liu *et al.* (1987) *J. Immunol.* 139:3521-3526; Sun *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:214-218; Nishimura *et al.* (1987) *Canc. Res.* 47:999-

1005; Wood *et al.* (1985) *Nature* 314:446-449; Shaw *et al.* (1988) *J. Natl. Cancer Inst.* 80:1553-1559; Morrison, S. L. (1985) *Science* 229:1202-1207; Oi *et al.* (1986) *BioTechniques* 4:214; Winter U.S. Patent 5,225,539; Jones *et al.* (1986) *Nature* 321:552-525; Verhoeyan *et al.* (1988) *Science* 239:1534; and Beidler *et al.* (1988) *J. Immunol.* 141:4053-4060.

[00277] An anti-16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 antibody can be used to detect 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the 16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 protein. Anti-16386, 15402, 21165, 1423, 636, 12303, 21425, 27410, 38554, 38555, 55063, 57145, 59914, 94921, 16852, 33260, 58573, 30911, 85913, 14303, 16816, 17827 or 32620 antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, □-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

[00278] This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application, as well as the Figure and the Sequence Listing is incorporated herein by reference.

EXAMPLES

EXAMPLE 1: TISSUE DISTRIBUTION OF USING TAQMAN™ ANALYSIS

[00279] This example describes the TaqMan™ procedure. The Taqman™ procedure is a quantitative, reverse transcription PCR-based approach for detecting mRNA. The RT-PCR reaction exploits the 5' nuclease activity of AmpliTaq Gold™ DNA Polymerase to cleave a TaqMan™ probe during PCR. Briefly, cDNA was generated from the samples of interest, *e.g.*, heart, kidney, liver, skeletal muscle, and various vessels, and used as the starting material for PCR amplification. In addition to the 5' and 3' gene-specific primers, a gene-specific oligonucleotide probe (complementary to the region being amplified) was included in the reaction (*i.e.*, the Taqman™ probe). The TaqMan™ probe includes the oligonucleotide with a fluorescent reporter dye covalently linked to the 5' end of the probe (such as FAM (6-carboxyfluorescein), TET (6-carboxy-4,7,2',7'-tetrachlorofluorescein), JOE (6-carboxy-4,5-dichloro-2,7-dimethoxyfluorescein), or VIC) and a quencher dye (TAMRA (6-carboxy-N,N,N',N'-tetramethylrhodamine) at the 3' end of the probe.

[00280] During the PCR reaction, cleavage of the probe separates the reporter dye and the quencher dye, resulting in increased fluorescence of the reporter. Accumulation of PCR products is detected directly by monitoring the increase in fluorescence of the reporter dye. When the probe is intact, the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence. During PCR, if the target of interest is present, the probe specifically anneals between the forward and reverse primer sites. The 5'-3' nucleolytic activity of the AmpliTaq™ Gold DNA Polymerase cleaves the probe between the reporter and the quencher only if the probe hybridizes to the target. The probe fragments are then displaced from the target, and polymerization of the strand continues. The 3' end of the probe is blocked to prevent extension of the probe during PCR. This process occurs in every cycle and does not interfere with the exponential accumulation of product. RNA was prepared using the trizol method and treated with DNase to remove contaminating genomic DNA. cDNA was synthesized using standard techniques. Mock cDNA synthesis in the absence of reverse transcriptase resulted in samples with no detectable PCR amplification of the control gene confirms efficient removal of genomic DNA contamination.

EXAMPLE 2: TISSUE DISTRIBUTION OF USING *IN SITU* ANALYSIS

[00281] For *in situ* analysis, various tissues, *e.g.*, tissues obtained from normal colon, breast, lung, and ovarian normal tissue, as well as colon, breast, lung, and ovarian tumors, colon metastatic to the liver, and angiogenic tissues were first frozen on dry ice. Ten-micrometer-thick sections of the tissues were post-fixed with 4% formaldehyde in DEPC treated 1X phosphate- buffered saline at room temperature for 10 minutes before being rinsed twice in DEPC 1X phosphate-buffered saline and once in 0.1 M triethanolamine-HCl (pH 8.0). Following incubation in 0.25% acetic anhydride-0.1 M triethanolamine-HCl for 10 minutes, sections were rinsed in DEPC 2X SSC (1X SSC is 0.15M NaCl plus 0.015M sodium citrate). Tissue was then dehydrated through a series of ethanol washes, incubated in 100% chloroform for 5 minutes, and then rinsed in 100% ethanol for 1 minute and 95% ethanol for 1 minute and allowed to air dry.

[00282] Hybridizations were performed with ^{35}S-radiolabeled (5 X 10^7 cpm/ml) cRNA probes. Probes were incubated in the presence of a solution containing 600 mM NaCl, 10 mM Tris (pH 7.5), 1 mM EDTA, 0.01% sheared salmon sperm DNA, 0.01% yeast tRNA, 0.05% yeast total RNA type X1, 1X Denhardt's solution, 50% formamide, 10% dextran sulfate, 100 mM dithiothreitol, 0.1% sodium dodecyl sulfate (SDS), and 0.1% sodium thiosulfate for 18 hours at 55°C.

[00283] After hybridization, slides were washed with 2X SSC. Sections were then sequentially incubated at 37°C in TNE (a solution containing 10 mM Tris-HCl (pH 7.6), 500 mM NaCl, and 1 mM EDTA), for 10 minutes, in TNE with 10μg of RNase A per ml for 30 minutes, and finally in TNE for 10 minutes. Slides were then rinsed with 2X SSC at room temperature, washed with 2X SSC at 50°C for 1 hour, washed with 0.2X SSC at 55°C for 1 hour, and 0.2X SSC at 60°C for 1 hour. Sections were then dehydrated rapidly through serial ethanol-0.3 M sodium acetate concentrations before being air dried and exposed to Kodak Biomax MR scientific imaging film for 24 hours and subsequently dipped in NB-2 photoemulsion and exposed at 4°C for 7 days before being developed and counter stained.

[00284] **Equivalents**

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.